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THE GENETICAL INTERPRETATION OF STATISTICS OF THE THIRD DEGREE IN THE STUDY OF QUANTITATIVE INHERITANCE

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For the past 20 years genetical methods have gradually been made more and more familiar to the practical breeders of plants and animals, upon whom the improvement for human use of the domesticated animals and cultivated plants finally depends. During this period it has become increasingly clear that the hereditary mechanism is well represented by the Mendelian scheme, as extended mainly by the work of the Drosophilists. It has been equally clear, however, that in all the practical problems of animal or plant improvement we are invariably faced with quantitative characters, which have shown themselves to be entirely intractable by the familiar genetical methods. These methods rest primarily upon the recognition of the effects of different single factors, and when these can be recognized the study of their effects in combination follows as a matter of routine. When individual factors cannot be recognized the analytic method of genetic study cannot even be commenced, and the question arises as to whether genetics as a science has any further resource to offer.

The successes of analytic genetics have been obtained mainly with the numerous deleterious recessives which are abundant in most species, with certain easily recognizable characters of practical importance to the plant or animal breeder and with fancy characters such as the crest, or silky plumage in the fowl, which, however attractive to fanciers, cannot be regarded as of general utility to mankind. The development of the quantitative characters on which practical utility is founded owes very little to genetic analysis except in so far as it has been demonstrated that it depends on a definite gene complex. This is implicit in the study of the individual effects of Mendelian factors without the means of evaluating the mass effects of a large number of minor factors severally influencing the utility character. We would stress, however, that the study of the metrical characters is not only of utilitarian interest. The nature of the heritable

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GENETICS 17: 107 Mr 1932

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elements which cause continuous or fluctuating variability must indeed be studied if progress in this direction is to be made possible; but such studies are also essential for an understanding of the evolutionary process by which organisms have been brought to their present state of organization and adaptation.

Most of the many workers who have attacked the problem of quantitative characters have carried their work far enough to verify that the heritable variability available was probably due to a large number of Mendelian factors, interacting generally in a cumulative manner. The principal criterion has been the greater variance of the F_2 as compared to the F_1 sample. These samples are, however, generally of very different magnitude, and care has not always been taken that the conditions of culture have been such as to make the comparison a valid one. It would seem essential for the purpose that the non-genetic causes of variability should be carefully equalized. With plants, the cultures to be compared should be grown in the same year, and if, as is probable, the F_2 is the larger culture, its variability should be estimated only within areas of the same size as that occupied by the F_1; for, with uniform seed, we can always obtain a higher variability by using a larger area.

In the case of this comparison of variance, a biometrical technique has been used to verify a genetical conclusion. In seeking for further points in the genetical situation which biometrical methods, combined with an adequate cultural technique, might be able to evaluate, it should be borne in mind that, as a school, the biometricians have shown themselves singularly unreceptive to genetical ideas. Methods which are genetically appropriate will not therefore be found ready made, and constants such as the correlation coefficient, which have been introduced with the highest biometrical testimonials, while they probably have, in suitable cases, an appropriate use, have assuredly done as much to confuse as they have to clarify the subject.

In studying the properties of a system of interacting factors it has been shown (FISHER 1918) that departures from the simple additive law of interaction will usually have effects somewhat similiar to non-heritable modifications. We may therefore be confident that, even if a strictly additive interaction is not exactly realized, the mass effects of segregation in a large number of factors will closely simulate those of simple cumulative systems. In such a system certain special quantities, of which the mean and the variance are examples, possess the remarkable property that each is simply compounded of contributions derived from the several factors acting singly. Thus the heritable variance observable among any group of

organisms may be regarded as the sum of the variances due to the individual factors. The portion of the variance which is heritable may be easily estimated from the covariances or mean products of the measurements of related individuals so that, without being able to recognize any single factor, we have a direct means of estimating their total contribution to the heritable variance.

This, however, is not enough to evaluate the selective potentialities of the population under examination. A number of further questions present themselves, most of which must at present remain unanswered. The same total variance might be contributed by a few factors each having a relatively large effect or by a multitude of smaller modifiers. In both cases progress can be made at once by selection, but whereas in the first case such progress will soon be accompanied by a decrease in the variance available, and will therefore soon be slowed down, in the second case progress can be continued much further in the same direction without the introduction of fresh material. Equally important, and fortunately less elusive, is the incidence of dominance; for mass selection will be far more successful in establishing recessives having a desirable effect than in establishing dominants of like effect, and this provides an obvious reason why, in material which has already, consciously or unconsciously, been much selected, the recessives are generally found to be variants in the direction which is judged to be disadvantageous.

We shall give some examples of the kind of data in which this bias in the prevalent direction of dominance, which selection must tend to introduce, appears to be shown. For the moment let us notice that effective biometrical methods of evaluating this bias will be of immediate practical value in the evaluation of the selective potentialities of a given population. From the purely scientific point of view it is also of importance that the dominance bias constitutes an existing record of the prevalent direction in which selection has acted in the immediate past. A geologist, by examining the population of individuals existing at a given horizon, might be able not only to specify the mean value and the variance of any measurement in this population, but might have a direct indication of the direction in which this measurement was in process of change.

STATISTICS OF THE THIRD DEGREE

In the study of the various methods by which the effects of biassed dominance may be brought to light, we shall be invariably led to the use of statistics of the third degree. Our knowledge of these quantities on the algebraic side is at present very incomplete. The birth of modern statistics

during the past generation may be typified by the transfer of attention from the totals and means characteristic of simple accountancy to the statistics of the second degree, the sums of squares and products, on which the whole apparatus of the calculus of correlations, or in more recent times of the analysis of variance and covariance, has been built. Statistics of the third and higher degrees have, of course, been used in fitting frequency curves and surfaces, but merely to evaluate empirical and arbitrarily chosen mathematical constants; and their practical inappropriateness for this purpose has been shown by their low efficiency in, for example, fitting the parameters of the Pearsonian curves. There is at present no comprehensive method of handling the statistics of the third degree analogous to the analysis of variance and covariance, to which nearly all work with second degree statistics can be reduced. Consequently, the methods we shall illustrate will probably be found to be capable of much improvement, and no exactitude can be claimed for the estimates of sampling error, or in consequence for the tests of significance. This, however, is a drawback which we may expect to be remedied with equal pace with the improvement of the experimental data, to which these methods may be applied.

In the case of statistics of the second degree we distinguish between the variance derived from the squares of the values of a single variate, and the covariance derived from the products of the values of the two different variates. The corresponding statistics of the third degree are of three kinds: (a) those derived from the cubes of the values of a single variate, (b) those derived from the product of one variate with the square of a second, and (c) those derived from the product of three different variates. It will be seen that all three types are, in different cases, of value. For each type we must throw our calculations in such a form as to obtain an unbiassed estimate of some parameter which, like the variance, satisfies the cumulative property, and which in consequence is interpretable in terms of the individual factors of the Mendelizing system.

For example, from a series of values of a single variate we can calculate the three statistics of the first, second and third degrees, namely,

$$k_1 = \frac{1}{n} S(x)$$

$$k_2 = \frac{1}{n-1}\left\{S(x^2) - \frac{1}{n} S^2(x)\right\}$$

$$k_3 = \frac{n}{(n-1)(n-2)}\left\{S(x^3) - \frac{3}{n} S(x^2)S(x) + \frac{2}{n^2} S^3(x)\right\}$$

where S( ) stands for summation over the sample observed, and n is the sample number, which are equivalent if $\bar{x}$ is the mean, to

$$k_1 = \bar{x}$$

$$k_2 = \frac{1}{n-1} S(x - \bar{x})^2$$

$$k_3 = \frac{n}{(n-1)(n-2)} S(x - \bar{x})^3.$$

Then it has been shown that k_1, k_2, k_3 are unbiassed estimates of quantities K_1, K_2, K_3 characteristic of the population sampled and possessing the cumulative property.

A. B. D. FORTUYN (1931, p. 163) gives eight seriations for the frequencies of different numbers of tailrings in different strains of mice, derived from *Mus musculus*, *Mus wagneri* and hybrids between these two forms. He was able to show that the variation in ring number was largely hereditary, for by selection from a common stock he obtained strains with average ring numbers, 142.6 and 216.2 respectively. Selection for high values of a variate should, when applied to a symmetrical population, generally shift the value of k_3 in the negative direction; equally, selection for low values should shift it in the positive direction. The amount of these changes will depend on the number of factors present. In an ideal case in which selection in opposite directions was applied to the F_2 from two homozygous lines, so that all pairs of allelomorphs were present initially in a 1:1 ratio, the ratio of the change in k_3 to that effected in the mean k_1 should be initially

$$\frac{-\frac{1}{2}S(d^4)}{S(d^2)}$$

where 2d is the difference between the homozygous forms in any one factor, and S stands for summation over the different factors. The rate at which the third moment is modified for a given change in the mean is evidently greater, other things being equal, the smaller the number of factors to the segregation of which the variance of F_2 is to be ascribed. As it stands it affords therefore a crude method of estimating or at least of setting a lower limit to the number of factors present. Although FORTUYN'S material was not formed as an F_2 from homozygous lines, it may be of interest to point out that the high selection line has in fact a negative k_3, though, on the number counted, not a significant value. Of the seven lines given, however, two, both with high ring numbers, do show significantly negative k_3, while a third with low ring number gives a k_3 which is significantly positive. The

phenomenon to be expected thus does not seem to be beyond attainable precision. The seven values are as follows:

TABLE 1

	k_1	k_3	g	n
Mus wagneri	138.8	−66.4	−.095±.228	113
L T M	142.6	−36.2	−.072±.229	111
B W T W	150.1	+2162.1	+.581±.199	149
W T W	159.0	+467.3	+.278±.182	179
Albino *Mus musculus*	189.3	−1194.0	−.526±.122	446
W M	195.0	−1667.6	−.584±.160	230
H T M	216.2	−238.5	−.258±.143	290

The best available test for the significance of k_3 (FISHER 1928) seems to be to calculate the ratio $g = k_3 k_2^{-3/2}$; then for sampling from a normal population the true variance of g is $6n(n-1)/(n-2)(n+1)(n+3)$ and its distribution is, for samples over 100, sufficiently near to normality for significance to be inferred from the standard errors as shown in the table. The standard error will be used throughout this paper in testing significance.

This example is illustrative only of the type of biometrical effect which is not beyond experimental precision, by which direct information may be obtained as to the distribution of the heritable variance among the genetic factors present. The interpretation of any particular body of data for which this effect was measured could evidently be carried much further by estimating also such quantities as the heritable variance and those unsymmetrical effects ascribable to dominance. These latter will indeed, in practice, almost always complicate the interpretation of any data bearing on the size and number of the heritable factors.

THE EFFECTS OF DOMINANCE BIAS ON F_3 PROGENIES

The observational facts that the cross (F_1) between two strains frequently shows greater "vigour," or growth rate, than either parental type and that inbred lines frequently show a falling off in size, which is reversible by a single cross, may be interpreted either on the view that there is among the genetic factors present a pronounced bias in dominance, in the sense that greater size is more usually dominant to less size, than *vice versa*, or, on the contrary, that the heterozygote in a single factor is frequently larger than either of the corresponding homozygotes. These two views differ considerably in their practical consequences, but the contrast may be reduced to the quantitative question of the normal position of the heterozygote in a single factor relative to the two homozygotes.

Let us suppose that two homozygotes differing in any one factor differ on the average in the metrical character under observation by a quantity 2d, so that the mean values for the two homozygotes differ from an arbitrary origin (the mid-point between the two homozygotes) by $+d$ and $-d$; we may then represent the average deviation of the heterozygote from the same origin by h. If h is generally positive, or at least generally positive for the more important factors, there will be in the system of factors considered a positive bias of dominance. The heterozygote for the whole group of factors will then exceed the mean of any two complementary homozygotes from which it might have been obtained. For any factor, if h lies between the limits $-d$ and $+d$, dominance will be partial or incomplete, if it is equal to $\pm d$ dominance will be complete, but if it exceeds $+d$ we shall have a case of superdominance. We shall consider how the biometrical data from F_3 progenies may be used to calculate whether the factors present as a whole have values of h which are positive, and if so whether there is evidence that they exceed the value d.

Although the main object of this paper is to call attention to the significance of various statistics of the third degree, yet it will be convenient here to state briefly some second degree results, which, though long known in principle, have not, we believe, been developed in a form convenient for experimental utilization. The three phases of any factor, if fully viable, may be expected in F_2 in the ratio 1:2:1. It easily follows that the contribution of such a factor to the variance in F_2 will be $\frac{1}{4}(2d^2+h^2)$, when the deviations are measured from the mean, $\frac{1}{2}h$. The total observable variance in F_2 does not, however, provide a satisfactory basis for evaluating directly the sum of these quantities, since, in all quantitative characters which are susceptible to environmental influences, a positive contribution will be made by environmental modification, and it does not appear that any experimental refinement could altogether eliminate this source of error. In the case of the covariance, on the other hand, the environmental deviations will be equally frequently positive and negative, and will only lower the precision of the result by increasing the quantities upon which the estimate of error is based. The covariance is calculated from $S(x-\bar{x})(y-\bar{y})/n-1$ where x and y are the two variates and n is the sample number. With much plant material two types of covariance may be fairly readily obtained: (1) the covariance between the F_2 parent and the mean of the F_3 progeny derived from it, the contribution of each independent factor to which is $\frac{1}{4}(2d^2+\frac{1}{2}h^2)$ and (2) the covariance of parent and progeny when the F_2 are crossed *inter se* at random, the value in this case being $\frac{1}{4}d^2$. From these two quantities we can, with precision limited only by the homozygosity of

the parent stocks, determine the part of the F_2 variance which is genotypic merely by taking twice their difference. What is equally interesting, the method shows a way of separately evaluating the ratio of the mean or average of the quantities h^2 to that of the quantities d^2, and so of discriminating between the hypothesis that the system of cumulative factors is one in which dominance is generally absent or slight, as is often assumed, and the hypothesis more generally favored when hybrid vigor is manifest that dominance is as complete in the quantitative factors as it generally is in factors which can be isolated for separate study.

Statistics of the second degree can obviously not distinguish whether h is positive or negative; they cannot therefore be used to investigate the extent to which dominance is biassed. Indications of this may be obtained from the first degree statistics, the means, as when an F_1 exceeds the mean of the parental values, and we infer that h is more frequently or more largely positive than negative. The comparisons of mean values, of which the most important is the difference between F_1 and F_2 is, however, a matter of some experimental difficulty, especially when the number of F_1 seeds is small, and, though they would be valuable in conjunction with other facts, by themselves they are not capable of more than a qualitative interpretation. From a set of F_3 progenies, however, it is possible to obtain three statistics of the third degree, which have a direct relevance. These are:

(1) The mean value of the k_3 from each of the progenies, to which each factor contributes $(-3/8)d^2h$.

(2) The covariance of the k_1 and k_2 in different F_3 progenies, to which each factor contributes $+h(2d^2+h^2)/32$.

(3) The k_3 of the means of different F_3 progenies, to which each factor contributes $(-3/8)d^2h$.

In respect of availability we shall show that no very extensive data are required to estimate the first of these quantities, while a larger number of progenies than in the examples to be given, with the exception of the barley data, though not an impossibly large number, would be needed to obtain good values for the second. The third would evidently be liable to large disturbances owing to the varying fertility of the areas upon which different progenies must be grown, and is in any case liable to much larger sampling errors than is the first. The first process will therefore always give the preferable value. What is important, however, is that, by a comparison of the mean value of k_3 with the covariance of k_1 and k_2, it is possible directly to distinguish between the views that an apparent effect of heterosis is due to ordinary dominance, either complete or incomplete, favoring the

larger values, in which case a homozygote may be established as vigorous as any heterozygote, and the alternative view that in many factors the heterozygote is more vigorous than either homozygote. If we multiply the covariance by 4 its value will be greater than, equal to, or less than the mean value of k_3 (with sign reversed) according as h (supposed positive) is greater than, equal to, or less than +d. With true superdominance four times the covariance should have a positive value exceeding the negative average value of k_3 within F_3 progenies, while if we are confronted only with a strong positive bias of the dominance, it should at most be equal to this value. If the system were of so simple a kind that in each factor the heterozygote was equal to the larger homozygote, we should find confirmation of the fact from the equality of $S(h^2)$ and $S(d^2)$, and should know that only by specific interactions could the average be raised above the level of the multiple heterozygote. Equally, if the covariance is less than this critical value, it is clear that the possibilities of mass selection have not been exhausted.

An example may be taken from the distribution of leaf length for 13 F_3 families of lettuce given by C. E. DURST (1930, p. 266). The mean leaf length in F_1 was greater than that of either parent, while the mean in F_2

TABLE 2

NUMBER OF INDIVIDUALS	k_1	k_2	k_3
21	4.286	3.014	1.427
8	6.000	13.143	4.572
39	7.590	1.143	−0.720
26	7.615	7.126	−2.381
17	7.647	3.742	−1.146
50	8.180	6.559	−3.122
25	8.480	10.927	−24.565
47	8.936	4.061	−2.660
13	9.539	5.102	−0.975
13	9.846	8.974	−20.700
35	9.886	8.104	−9.715
53	10.585	2.786	−5.631
11	12.000	8.200	−18.333

was slightly shorter than the larger leaved parent; by this indirect comparison the mean of F_2 may be judged to be about 2.2 units less than in F_1, one unit being 1.5 cm. The numbers of individuals and the values of k_1, k_2 and k_3 obtained for the 13 F_3 progenies are shown in table 2, differences of 1.5 cm being taken as one unit, the first unit being at 10 cm.

From these we find directly the mean value of k_3 to be -6.458 ± 2.532, a negative value, as the theory has indicated, which is statistically significant, but owing to the small number of families, not well determined numerically. For the covariance of k_1 and k_2 we have $+0.492$, which is, in accordance with the theory of cumulative factors, positive, though no statistical significance is to be attached to this fact as its standard error is as high as 2.017.

The k_3 of the means of the thirteen F_3 lines is found to be -3.476 ± 6.159, a negative value, again in agreement with the theory but not significant. The sampling variance of the mean k_3 has been obtained from the formula $\{1/(n-1)n\}\ S(k_3-\bar{k}_3)^2$, where n is the number of k_3's calculated, the variance of the k_3 of the F_3 means from $\{6n/(n-1)(n-2)\}\ k_2^3$ and the variance of the covariance of k_1 and k_2 from $(1/n-1)\{V(k_1)V(k_2)+V^2(k_1k_2)\}$ where $V(k_1)$, $V(k_2)$ stand for the estimated variance of k_1 and k_2 in different families and $V(k_1k_2)$ for their estimated covariance.

It will be observed that very different numbers of plants were obtained in the different F_3 families, and consequently the precision of the statistics derived from them must be expected to vary greatly. We may anticipate in general that the best possible theoretical estimates will be something between those obtained by giving, as above, equal weights to all families, and the corresponding values obtained by weighting each in proportion to the number of plants recorded. To ascertain whether in this case weighting would give appreciably increased precision, the values were recalculated on the latter system. The standard error of the mean k_3 was reduced from 2.532 to 2.087, and that of the covariance from 2.017 to 1.427, showing that it will be found a decided advantage in the use of such data for our present purpose if the F_3 progenies are approximately equal in number.

This advantage is emphasized equally by the circumstance that the statistics derived from the means of a limited number of plants will be affected by the k_3 of their distribution about their means. Both the covariance and the k_3 of the means require for this reason a correction algebraically equal to $-\bar{k}_3/s$ where s is the number of plants per family. With variable family numbers it will doubtless be sufficient in applying this correction to use the harmonic mean of the actual numbers, but the existence of a correction of this kind is a sufficient reason for keeping the numbers of the F_3 families as nearly constant as is conveniently possible.

The fourfold value of the covariance of k_1 and k_2 is here less than the mean k_3, indicating that h is generally less than $+d$; this is true even when the considerable correction $+.360$ is added to the crude value $+.492$. No significance can, however, in this case be attached to the comparison, con-

sidering the magnitude of the standard errors. It is of interest to note also that the k_2 of the F_2 progenies, 9.536, was almost twice that of the weighted k_2 of the F_3 progenies, that is, 5.468. The contribution of a single factor to the variance of the former would be $\frac{1}{4}(2d^2+h^2)$, and to the latter $\frac{1}{8}(2d^2+h^2)$. The comparison of the two variances thus suggests that a large proportion of the observed variance was in both cases genetic in origin.

For a critical study far more extensive data would be needed. The data given here are recognized to be inadequate except to point out how the problem may be attacked. Not only would more plants be required in F_2 and the F_3 lines but the number of F_3 lines should also be increased greatly in order that these should adequately sample the segregation in F_2. With the use of sufficiently extensive data it should be possible to reduce the standard errors to limits which would permit of exact comparisons between the different statistics and to fix the prevailing ratio of h and d within reasonable bounds. Replication, as a means of reducing the effect of soil variation, would be highly desirable.

Another example will be taken from published data on inheritance of a quantitative character in maize. EMERSON and EAST (1911, p. 77) presented data on inheritance of height of maize plants from a cross of Tom Thumb pop and Missouri dent, two open pollinated varieties. A guess mean was taken at 18 dcm, 1 dcm was taken as a unit and the following table computed from the sixteen different F_3 distributions given:

TABLE 3

NUMBER OF INDIVIDUALS	k_1	k_3	k_4
40	−7.225	2.076	0.597
114	−5.114	6.102	3.145
64	−6.016	2.524	0.106
65	−0.785	2.734	−3.155
90	2.011	4.685	−2.975
85	0.306	6.310	2.698
82	1.488	6.944	−4.716
85	0.635	6.162	−7.731
82	1.598	3.503	−0.268
95	4.432	5.567	−1.393
87	1.805	5.182	−5.226
149	3.745	3.070	−0.657
87	4.207	5.422	−2.954
93	4.774	4.764	4.943
87	5.862	2.981	−0.162
76	7.145	5.512	−0.011

The k_2 of the F_2 was 12.499 ± 1.415 and the k_3 of the F_2 was -15.694 ± 8.666. From the table we find the mean k_2 of the F_3 lines to be 4.596 ± 0.389 and the mean k_3 of the F_3 to be -1.110 ± 0.821. The latter is negative as expected for a dominance bias, but cannot be considered statistically significant with the data available. The covariance of k_1 and k_2 is found to be $+1.925 \pm 1.795$, a positive value as expected, but again not significant. Twice the value of the k_2 of the F_3 is here even less than the k_2 of the F_2. The variance of the mean k_2 of the F_3 lines was obtained from the formula $\{1/n(n-1)\}S(k_2-\bar{k}_2)^2$ where n is the number of F_3 families.

In the summary of the paper by EMERSON and EAST we find a statement relative to the "lack of skewness in the F_2 frequency distributions" for the crosses employed in studies on inheritance of height of plants. Taking the total distributions for F_2 in tables 25, 26, 27, 28 and 30 and the same for the F_2 in 1911 in table 29 we may determine statistically whether these distributions were or were not symmetrical. We find that $(k_3 k_2^{-3/2})$ was -0.105 ± 0.120, -0.254 ± 0.096, 0.028 ± 0.161, 0.012 ± 0.105, -0.355 ± 0.192 and -0.161 ± 0.106 in tables 25 to 30, respectively. A negative bias is indicated in four of the six crosses tested; although in the second alone is the skewness statistically significant, yet two other negative values are suggestively large.

Early studies of heterosis emphasized the fact that if dominance of growth factors were the explanation of hybrid vigor the F_2 distribution would be skew. Such was not the case, it was argued. With a large number of growth factors the F_2 distribution would tend toward the normal, and large numbers would be needed to demonstrate a negative bias. That such a negative bias is fairly common is demonstrated by the negative k_3 found from the data examined in this paper.

The maize data were obtained from a cross of two open pollinated varieties which were undoubtedly heterozygous in many of their factors for height. The data are given only to show how the problem may be attacked. Maize offers unusual possibilities for biometrical studies on quantitative inheritance. Some of the advantages of maize will be enumerated.

Selfed lines are already available which may be considered homozygous for the greater part of their growth factors. Crosses are easily made and a large number of seeds usually obtained. It would be possible, usually, to self a sufficient number of F_2 plants to obtain seed for tests in F_3 and to leave sufficient seed of the F_2 generation for comparison of the F_2 with the F_3 progenies in the following year. Since maize is very highly cross pollinated, seed from the various F_2 plants may be planted in an isolated plot also and allowed to cross *inter se*.

Parent lines, F_1, F_2, F_3 and lines from F_2 plants crossed *inter se* can then be grown in a single year in a replicated yield trial. The value of careful replication cannot be over-emphasized for such a study as a means of eliminating soil variation. Until more information is available on this point it may be suggested that, say, 50 to 100 plants in each of 100 F_3 lines ought to give sufficient data for a critical study. These might well be grown in five replicated plots of 10 to 20 plants each.

The contribution of a single factor difference to the following statistics may be noted:

Statistics of the second degree

Variance of F_2	$\frac{1}{4}(2d^2+h^2)$
Mean variance of F_3 progenies	$\frac{1}{8}(2d^2+h^2)$
Variance of means of F_3 progenies	$\frac{1}{4}(2d^2+\frac{1}{4}h^2)$
Covariance of F_2 parental value with mean of its F_3 offspring	$\frac{1}{4}(2d^2+\frac{1}{2}h^2)$
Covariance of F_2 parental value with mean of its biparental offspring	$\frac{1}{4}d^2$
Mean variance of biparental progenies	$\frac{1}{16}(4d^2+3h^2)$
Variance of means of biparental progenies	$\frac{1}{16}(4d^2+h^2)$
Mean variance of maternal progenies	$\frac{1}{8}(3d^2+2h^2)$
Variance of means of maternal progenies	$\frac{1}{8}d^2$

The biparental progenies are those obtained by crossing two F_2 plants. The maternal progenies are taken to be the progenies of plants exposed to open pollination by sister F_2 plants. Whether the multiplicity of measures also affords a method of measuring and eliminating the effects of incomplete linkage, we have not investigated; it would seem premature to attempt this until biometrical studies with visibly classifiable factors have shown what kind of disturbance is to be looked for.

Of the above statistics the four variances of individual values will usually be sensibly increased by environmental modification; the variances of the means could be freed from this bias, if the progenies are not grown on separate areas. The two covariances should be free from bias, and the experimental arrangement could be devoted solely to diminishing their sampling errors.

Statistics of the third degree

k_3 of F_2	$-\frac{3}{4}hd^2$
Mean k_3 of F_3 progenies	$-\frac{3}{8}hd^2$
Covariance of means and variances of F_3	$(h/32)\ (2d^2+h^2)$
Covariance of F_2 parental value and variance of F_3	$(h/16)\ (2d^2+h^2)$
k_3 of means of F_3 progenies	$-\frac{3}{8}hd^2$
Mean k_3 of biparental progenies	$-\frac{3}{16}hd^2$
Covariance of means and variances of biparental progenies	$-\frac{1}{8}hd^2$
Covariance of parental values and variances of biparental progenies	$-(h/32)\ (2d^2-h^2)$
Covariance of biparental progeny and biparental product	$-\frac{1}{8}hd^2$
k_3 of means of biparental progenies	$-\frac{3}{16}hd^2$
Mean k_3 of maternal progenies	$-\frac{3}{16}hd^2$

Covariance of means and variances of maternal progenies	$-\frac{1}{8}hd^2$
Covariance of parental values and variances of maternal progenies	$-\frac{3}{16}hd^2$
k_3 of means of maternal progenies	0

It will be noticed that the same quantities may be obtained experimentally in many different ways. This should afford a valuable empirical test as to the consistency of the genetical interpretation and will also prove valuable in detecting and eliminating the metrical bias, to be discussed later, with which statistics of the third degree, apparently with the exception of the covariance of the biparental progeny mean with the biparental product, may be affected. For the moment we need only notice that since this bias is always of the same sign, significant values of opposite signs cannot but indicate genuine genetical effects.

Some of these comparisons will be subject to greater errors than others. The previous examples show that the mean k_3 of F_3 lines is determined with much greater precision than the k_3 of the means, or the covariance of k_1 and k_2. One of the objects to be aimed at in exploratory work of this kind must always be to ascertain which biometrical values, of those which have an important genetical interpretation, can be ascertained in practice with a useful degree of precision.

In any case when both parental values are known as well as the mean of the progeny, the effect of this negative correlation of the progeny with the parental product may be exhibited by a purely biometrical procedure, one in which the relationships of the different parent stocks are not taken into consideration. We may in fact always calculate the regression of progeny values upon the three "independent" variates, maternal value, paternal value and parental product. Such a partial regression equation was calculated from data on row number from 46 backcrosses of maize kindly supplied by E. W. LINDSTROM. From data on row number of both parents and mean row number of the progeny the partial regression of mean progeny row number was found to be given by $0.4418x + 0.5148y - 0.0364p$ where x and y are the maternal and paternal row numbers and p is the parental product. The covariance of mean of progeny on the product of parental deviations was negative as expected, but the value shown for partial regression was not significant, having in this case a standard error $\pm .0369$. The regression of progeny on mother or father was about one-half as expected.

There are, in general, two important sources of disturbance in biometrical studies concerned with a study of quantitative inheritance. The first may be termed the dominance bias. When dominance favours the larger values the segregating generations will tend to have a negative skewness

(as measured by k_3). The extent of this bias will depend solely on whether dominance is complete or incomplete. The maximum skewness would be obtained when dominance is complete. With h greater or less than +d this skewness would be reduced. It is from the properties connecting h and d in different types of distributions and matings that these quantities may be estimated, as shown previously.

Another source of bias is encountered also very frequently. This may be termed the metrical bias, since it depends on the scale of measurements used. It is a not infrequent observation that the standard error of yield of a group of plots on a field of low fertility is often higher than that of plots on a high fertility field. The yields of plots from a low fertility field vary more with slight variations in soil fertility than do plots on a field which is already producing nearer to its maximum. The k_3 of yields from a group of plots varying in fertility would, in such cases, be negatively skew.

An example of this "inherent" negative bias will be taken from measurements of height of barley plants grown in different plots, with different nitrogen fertilizers, at the ROTHAMSTED EXPERIMENTAL STATION in 1928. The heights of sixteen plants were measured in each of 24 plots, the plants being selected at random within each plot. The mean height in cm to the auricle of the last expanded leaf for all observations was 37.6175 cm. The mean k_2 for the 24 plots of 16 plants each was 67.107 ± 2.078 and the mean k_3 of the same 24 plots was -194.099 ± 61.231. The covariance of k_1 and k_2 was -14.369 ± 13.248. A negative skewness is evident, indicating an inherent negative metrical bias. The covariance is also negative, again indicating the same type of disturbance. It is not significant, however. Since a normal variety of barley was used, and barley is very highly self fertilized, this bias cannot be attributed to genetic segregation for plant height factors.

These two sources of bias, the dominance bias and the metrical bias, will tend to counteract one another in the covariance of k_1 and k_2 of the lines in genetical studies of quantitative inheritance. The extent of the disturbance due to the metrical bias will depend somewhat on which statistics are used. It will be greatest in the covariance of k_1 and k_2 and will not be as serious a source of disturbance to the mean k_3.

Extensive data on height of barley plants from a cross of a two row (*Hordeum distichum nutans*) and a so-called six row variety (*Hordeum tetrastichum*) made by TEDIN and grown at Weibullsholm, Sweden, in 1930, will be used to illustrate the type of information which may be obtained from such data. This study was made entirely on F_2 material. Two hundred and seventy F_3 lines, segregating for the factors two row versus six row were

grown and individual height measurements obtained on 14,759 plants in these lines. The number of plants varied considerably in the different lines but weighting was not attempted.

The mean height of all the F_3 plants was 87.4 cm. Taking 5 cm as unit the mean k_2 of the 270 F_3 lines was 3.475 ± 0.077 and the mean k_3 -2.176 ± 0.204, a negative quantity in accordance with the theory that dominance of factors for plant height should give a negative k_3. This quantity was highly significant owing to the ample data on which it was based. Another third degree statistic was also available, that of the k_3 of the means of the F_3 lines. This was found to be -0.932 ± 0.567, a negative quantity as expected but not statistically significant. Since replication of the F_3 lines was not used this quantity would not be expected to be determined very accurately. The third statistic of the third degree calculated was the covariance of k_1 and k_2. This was found to be -0.564 ± 0.124, a negative quantity that was highly significant. If the dominance bias had been operating alone the covariance should be positive. The question arises as to whether the clearly significant negative value for the mean k_3 is to be ascribed to the co-operation of both measures of bias, or principally or entirely to the metrical factor.

For moderate bias it may be anticipated that the bias in the mean of the k_3 of the progenies will be proportional to six times the mean square of their variances, that is, to 81.8478, while the corresponding bias in the covariance of the means and variances of the families will be proportional to four times the product of the mean variance of the progenies and the variance of their means. This comes to 33.6060 or more than one-third of that found for the mean of k_3. Since the negative value found for the covariance, when corrected, as before explained, for the limited number measured in each family, is just less than a quarter of the mean value of k_3, we may conclude that the negative value of the covariance may be wholly accounted for by metrical bias without abolishing at the same time the negative values for k_3. In this case, however, it is clear that metrical bias has been a major factor in both values, and even the ample material measured would not allow us to attach significance to the residual genetic effects remaining after the metrical bias had been removed. It would, of course, be not surprising with a normally self fertilized plant, such as barley, that the pronounced bias in the dominance of genetic factors, comparable with that indicated for maize, should be absent or inconspicuous.

The average height of the 2 row, heterozygous (that is, for the 2 versus 6 row factor pair) and 6 row plants in the F_3 lines segregating for the 2 versus 6 row factor pair was found to be 88.2405, 88.0035 and 83.1190 cm,

respectively. The heterozygous group of plants cannot be considered significantly different in height from the 2 row plants. Apparently, therefore, as far as height factors linked with the 2 versus 6 row factor pair are concerned, no evidence of superdominance could be found. It seemed of interest to determine next the average variance (k_2) of height of the 2 row, heterozygous and 6 row plants when these were segregates in the same F_3 lines and grown on the same areas of land. The average k_2's were 87.6675, 84.1100 and 70.9825 square cm, respectively, for these three groups. The mean k_2 of the 2 row plants was 3.5575 ± 3.2275 square cm greater than the k_2 of the heterozygotes and 16.6850 ± 3.5950 square cm greater than that of the 6 row plants. The mean k_2 of the heterozygotes was 13.1275 ± 2.6500 square cm greater than the k_2 of the 6 row plants. The first of these differences is not statistically significant but the latter two are. When, however, the standard errors ($\sqrt{k_2}$) were expressed in percent of the mean heights, values of 10.61, 10.42 and 10.14 percent were obtained for the 2 row, heterozygous and 6 row groups. Apparently when the standard errors of the heights of these three classes of segregates, grown on the same small plots of land, were expressed in percent of the mean height the coefficient of variability was practically constant for the three groups.

SUMMARY

1. A genetical interpretation is given for various second and third moment statistics which are of use in studying quantitative inheritance.

2. Published data taken from lettuce and maize, and unpublished data from barley crosses are used to illustrate how the problem may be attacked. The special needs of data adequate for this purpose are illustrated, and certain possible precautions in planning the experiments are pointed out.

3. A study of the skewness of seven distributions for strains of mice selected for high and low tailring number indicated that the theoretical negative association between the statistics k_1 and k_3 in selected strains could probably be evaluated.

4. Formulae are given by which the effect of the dominance bias in the heterozygote in relation to the measurable characters of the homozygotes in F_2 or F_3 distributions or various types of crosses may be calculated.

5. The two common sources of bias (metrical and dominance) are discussed and data from a barley cross used to illustrate the results obtained when the former is of major importance.

6. Since the combined effect of the dominance and metrical biases may be obtained experimentally in many different ways, an empirical test of

the consistency of the genetical interpretations is available, as well as an opportunity of evaluating and eliminating the metrical bias.

7. Standard errors are given for the different statistics used.

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POLLEN GRAIN STUDIES AS AN INDICATION OF FERTILITY IN HYBRIDS

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Several investigators have reported evidence indicating a correlation between the percentage of normal pollen grains and the ultimate fertility of hybrid plants. If such a correlation were established it would prove valuable in the study of hybrid progenies comprising large numbers of plants. This is especially true of species of Compositae where under the best of conditions examination of meiotic divisions is handicapped by comparative scarcity of pollen mother cells at the desired stage. Consequently any data permitting ready estimation of the more fertile hybrids would reduce the amount of cytological preparation, and, under certain conditions, might dispense with it. This would be true of hybrids tending to exhibit quadrivalence in varying degrees and where pronounced lack of uniformity in size of homologues makes it impossible to interpret certain meiotic units as quadrivalents of smaller homologues or bivalents of larger ones.

In a previous investigation, the writer noted that in many selfed backcross hybrids of the cross *Crepis rubra*×*C. foetida* the percentage of good pollen grains tended to increase directly with the degree of return of the hybrids of this generation to the parental chromosome constitution (POOLE 1931). It was also found that amphidiploid (allopolyploid, didip-

TABLE 1

Percentage of good pollen.

PARENT SPECIES	F_1 (AMPHIHAPLOID)	AMPHIDIPLOID
S. nigrum, 90, ×*S. luteum*, 75–80	8	35.0
C. tectorum, 95, ×*C. capillaris*, 4.6–94.3	About 1	32.4
C. foetida, 96, ×*C. rubra*, 84.5	2.7	34.5

loid) plants of the same cross possessed higher percentages of good pollen than the F_1 or amphihaploid plants and consequently exhibited a greater degree of fertility. Other investigators have reported pollen grain counts in amphidiploid hybrids showing remarkably close agreement with these data. Data from JORGENSON (1928) and HOLLINGSHEAD (1930) may be cited.

In a study of hybrids between certain Crepis species BABCOCK and J.

CLAUSEN (1929) show that the closer the phylogenetic relationship between the parents the greater the degree of fertility and the higher the percentage of good pollen grains in the hybrids.

As indicated in table 1, HOLLINGSHEAD found considerable fluctuation in pollen counts in the x strain of *C. capillaris* from which her amphidiploid was obtained. Her detailed study indicated little difference in the percentage of good pollen grains from day to day for the same plant. The great differences found, however, were between different plants, and those exhibiting the lowest percentages of good pollen were those exhibiting the highest degrees of meiotic irregularity. This x strain of *capillaris* is known to have been derived from an open pollinated plant, and was therefore heterozygous.

Some investigators have criticized the use of pollen grain studies for this purpose on the ground that daily pollen fluctuations in flowers on the same plant were high. Furthermore, asynaptic genes, such as described in maize by BEADLE (1930), were properly cited as illustration of the fact that irregularity in pollen production is not in itself an indication of hybridity. In this latter instance, however, the effect of the asynaptic gene is so pronounced that when it occurs in homozygous material there can be no confusion with hybrid phenomena.

The following study was prompted by the discrepancy between these criticisms and the findings above reported by the Crepis investigators.

POLLEN STUDIES IN PURE SPECIES

Pollen grain studies were essayed on certain Crepis species flowering at Berkeley during the winter months. For this purpose Belling's iron-aceto-carmine stain was used. The heads of most Crepis species flower gradually, that is, on the first day of anthesis only the outermost circle of florets opens. The characteristic erect position of the style-branches of newly opened florets is unmistakable. Hence 6–8 new florets are easily removed daily from the head with a pair of forceps and placed in a drop of aceto-carmine into which the pollen grains immediately scatter of their own accord. The florets are then removed and the aceto-carmine drop is sealed in the ordinary manner. If a highly concentrated stain is used the slide is ready for counting within two or three hours.

Plants of four Crepis species were available, one plant each of the three species *mungieri*, *montana* and *laciniata*, and three plants of *leontodontoides*. From some work done by the writer several years ago it was suspected that fluctuations in daily temperature might influence pollen production. To provide the essential data a thermograph was installed in the same section

of the greenhouse with the plants. The temperatures used were the computed daily mean temperature, and the observed minimum night temperature. The temperatures and the estimated pollen grain percentages were then plotted on coordinate paper. Such graphic portrayal of the variations in temperature and pollen grain percentages was analyzed for any possible correlation. Sharp peaks were noted in temperature, especially the minimum temperature, but no peaks corresponded with the peaks of lesser degree disclosed by the pollen counts.

However, the history of pollen grain variation as shown by these curves indicated that the percentage of good pollen was relatively low at the beginning of the flowering cycle, reached a steady and comparatively high rate for the middle period of development, and dropped again to a low fig-

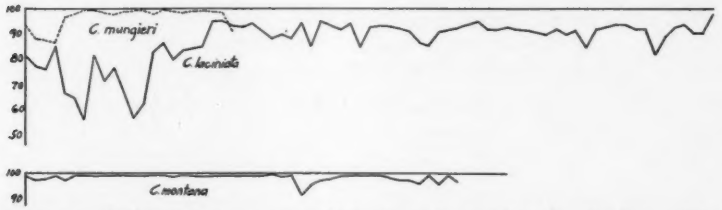

FIGURE 1.—Pollen percentage curves of 3 Crepis species (not flowering simultaneously). *C. mungieri* and *C. laciniata* were studied at beginning of flowering; *C. mungieri* and *C. montana* died during period illustrated.

ure for the few days at the termination of the cycle, that is, before the death of the plant. This state of affairs was shown in the curves of the two species obtained at the start of the flowering cycle, that is, *mungieri* and *laciniata*, and for the termination of the cycle for the two species dying during the course of the study, that is, *montana* and *mungieri*. *Crepis laciniata* had a long period of flowering, from January 15 to late in June, but study was discontinued after March 26, when sufficient information had been accumulated and the flowering of large numbers of hybrids had commenced.

From the *laciniata* data shown in figure 1 it is evident that the degree of variability found during the first two weeks is not repeated during the subsequent five or six weeks. The data, expressed statistically, show the following:

	Average percentage of bad pollen	*Coefficient of variability*
First 2 weeks	26.9	37.5
Next 3 weeks	9.0	29.3
Total time	15.4	69.6

In short, some distinction should be drawn between pollen counts made during the first two weeks of flowering and those obtained after the physiological mechanism has become adjusted to flowering. Such variability at the beginning of flowering has been noted by Nicotiana workers who frequently find that the first flower to appear is abnormal in many respects.

During the period of investigation of *laciniata* pollen three plants of *C. leontodontoides* came into flower. Consequently material was on hand for a more critical investigation of any possible environmental factor influencing pollen production. With pollen counts from four plants representing two species, any external factor or factors affecting pollen would be expressed in a definite correlation. From March 10 to March 26 determinations were made for all four plants, and correlation was computed as follows:

laciniata-leontodontoides No. 1, $r=0.0195\pm0.1740$
laciniata-leontodontoides No. 2, $r=0.1507\pm0.1596$
laciniata-leontodontoides No. 3, $r=0.2835\pm0.1502$
leontodontoides No. 2 *leontodontoides* No. 3, $r=0.2774\pm0.1508$
(correlation of *leontodontoides* No. 1 with Nos. 2 and 3 superfluous)

Hence it is clear that here no environmental factors have influenced the percentage of bad pollen to the same degree either in plants of the same or of different species. Furthermore, for several days separate heads of the same plant were sampled for percentage of bad pollen with the following results:

	C. leontodontoides No. 2			
	a	*b*	*c*	*d*
March 29	0.8	1.1	0.5	0.8
March 30	0.4	0.7	1.1	1.2
March 31	..	..	1.3	1.5
	C. laciniata			
March 30	6.0	8.2	4.4	7.7
March 31	9.4	9.3	6.5	9.1

The letters indicate the same heads, and it is seen that the variation from head to head on the same plant is no greater than is expected from errors in sampling.

The evidence thus far considered would indicate that, at least for pure species, the percentage of bad pollen is very little, if at all, influenced by external factors, and that the greatest range of variation within the flowering history of the plant is associated with the physiological adjustments to normal functions.

POLLEN STUDIES IN HYBRIDS

The material available for pollen grain studies in hybrids consisted of

two generations of plants derived from selfing the *C. rubra*×*C. foetida* amphidiploid referred to in the Introduction. The main point at issue is, if hybrid plants of the same generation differ in genetic constitution can the more fertile hybrids be discovered by a study of their pollen grain percentages? LAMMERTS (1931) found that among the derivatives of an amphidiploid *Nicotiana rustica N. paniculata* plant, four stable derivative lines were eventually obtained from selfing. His Nicotiana amphidiploid corresponded in many respects with the Crepis amphidiploid now under consideration. Both plants were highly sterile, and a study of meiosis indicated that in each of them the interspecific homologues tended to form quadrivalent chromosomes, although with variable frequency. It was this variable conjugation, apparently, that accounted for a large portion of the sterility. Consequently the discovery of stable derivative lines in the progeny of the Nicotiana plant pointed to a similar possibility in the progeny of the Crepis plant.

With this in mind it became important to identify the more fertile Crepis hybrids for purposes of isolation and subsequent selection. At the time of maturity the amphidiploid produced 55 normal achenes from an estimated potential number of 2037. Forty-five of these achenes germinated, comprising the F_3 generation. The percentage of good pollen grains for each plant was estimated when the first head flowered. At maturity all achenes were gathered, and the percentage of fertility was computed. Forty-four of these plants reached maturity thus permitting correlation calculations for a fair-sized population. As a result it was determined that the coefficient of correlation was 0.5357 ± 0.073. Twenty-six plants proved more or less fertile and eighteen were completely sterile.

The somatic chromosome garnitures of the entire population were carefully studied, as a result of which some interesting light may be thrown on sterility. This phase of the investigation will not be treated in this paper, however, our interest being chiefly with the bearing of pollen grains on fertility.

Several of these F_3 plants were considerably more fertile than the amphidiploid, as will be shown in a selected list of the more fertile plants included in the following table. In an attempt to establish stable lines the two most outstanding plants of F_3 were chosen, together with a third plant which had one chromosome extra, as parents of F_4.

Of course no attempt is being made in this small sample to give an impression of the total range of fertility. Some plants that proved completely sterile had quite high pollen counts, but none with low pollen counts proved very fertile, as can be seen from their omission above. One plant,

B 12, having 37.0 percent good pollen, died suddenly when only four heads had flowered. On inspection, however, some achenes were found to have commenced development. Two more plants had sufficiently high pollen counts to warrant the expectation of some fertility but failed to produce any achenes. Consequently it is evident that not all plants failing to produce achenes were genetically sterile. The fact that such a high correlation coefficient was obtained does indicate, to my mind, that the major cause of sterility is the genetic constitution. This belief is further strengthened by the data gathered from F_4, now to be considered.

TABLE 2

Most fertile F_3 plants.

PLANT NUMBER	PERCENTAGE GOOD POLLEN	PERCENTAGE FERTILITY
B 7	56.2	6.71*
B 9	28.0	3.45
B 11	21.4	1.27*
B 13	34.4	4.08
B 18	42.4	8.56*
B 19	27.2	2.13
C 7	35.3	2.22
C 14	22.4	1.26
C 15	47.0	1.93
F_2 Parent	34.5	2.73

* Plants used as parents of F_4

THE F_4 POPULATION

In consideration of the character of the pollen curve drawn from the data furnished by *C. laciniata*, figure 1, it was decided to take two samples of pollen, three weeks apart, from the plants of F_4. Consequently two sets of pollen counts were available for study before fertility data were computed.

It was evident at first glance that the general trend of these two sets of pollen counts was in strong agreement for individual plants. Some instances were found where a plant showed a somewhat higher percentage on the first count than on the second, or *vice versa*, but these were comparatively rare as will be seen from the correlation obtained from a study of the progeny of 30 B7.

Progeny	*Number of plants*	*Coefficient Correlation*	*Variability-X*	*Variability-Y*
31 Z 7	70	0.7208 ± 0.0388	48.2 percent	40.9 percent

The average percentage for the samples taken during the third week was slightly lower than for that taken during the first week, but the coefficients

of variability for the two sets of percentages were 48 and 41 respectively, with a very high degree of correlation between them.

From the outset all F_4 plants exhibiting good pollen counts of 30 percent or better were isolated from cross pollination in mosquito netting cages. The F_3 population apparently suffered no harm from this practice, but the occurrence of a week of unusually warm days killed a considerable number of these F_4 caged plants, with no noticeable injury to uncaged plants in the same section of the greenhouse. This misfortune doubtless accounts for much of the failure to obtain an improvement in fertility in F_4 comparable to that shown by F_3 over the F_2 parent, since all the more promising plants were undoubtedly affected. On the other hand, since the pollen grain counts themselves failed to exhibit the desired magnitude of improvement, there may have been no grounds for expecting the fertility to do so.

Nevertheless, a study of the correlation between pollen counts and fertility percentages warrants the belief that if these plants had not been caged some of them would have greatly exceeded the fertility of any F_3 plants. This belief is founded on a comparison of the coefficients obtained by treating data for caged and uncaged plants separately. Coefficients for the total number of plants, caged and uncaged, were statistically significant. Coefficients for the caged plants only, however, exhibited no statistical significance whatever, and when uncaged plants alone were correlated a marked improvement is made in the coefficients obtained.

TABLE 3

Correlation coefficients percentage good pollen—percentage fertility, F_4.

PROGENIES	COEFFICIENTS	NUMBERS STUDIED
31 Z 7	0.3635±0.0642	83
31 Z 11	0.3618±0.0963	37
31 Z 18	0.4216±0.0513	117
Total	0.3827±0.0374	237
CAGED PLANTS		
31 Z 7	0.1728±0.1690	15
31 Z 11	−0.9772±0.0218	3
31 Z 18	−0.1746±0.1427	21
Total	−0.0789±0.1074	39
UNCAGED PLANTS		
31 Z 7	0.3867±0.0696	69
31 Z 11	0.3809±0.0986	34
31 Z 18	0.4499±0.0549	96
Total	0.4019±0.0402	198

In the following tabulation of the correlation coefficients of F_4 the three progenies arising from each of the three F_3 parents are treated separately.

Hence there is justification for the belief that had all plants been treated alike, as they were in F_3, some individuals would have exceeded the estimated fertility of the F_3 parents giving rise to this population. Such a view is strengthened by a consideration of certain individuals. Three uncaged plants in progeny 31 Z 7 exceeded the fertility of their parent, although their percentages of good pollen were considerably lower. At the same time certain caged plants, especially three in progeny 31 Z 18, gave every indication from their pollen content of as high or higher fertility than any uncaged plant. None of these caged plants, however, realized their promise, despite the fact that the high pollen count was verified by two separate determinations.

TABLE 4

Comparison of some caged and uncaged plants.

PLANT NUMBER	PERCENTAGE GOOD POLLEN	PERCENTAGE FERTILITY
(Uncaged)		
31 Z 7.30	24.2	7.6
31 Z 7.63	41.3	7.3
31 Z 7.73	23.8	9.0
(Caged)		
Parent (30 B 7)	56.2	6.7
Parent (30 B 18)	42.4	8.6
31 Z 7.62	40.1	0.2
31 Z 18.11	41.4	2.1
31 Z 18.63	44.4	0.4
31 Z 18.81	40.2	0

The exceptional nature of these figures will be better appreciated if it is borne in mind that the F_4 population as a whole exhibited statistically significant correlations, uniform for all the separate progenies, as shown in table 3.

DISCUSSION

Since the first adequate description of an experimentally obtained amphidiploid in 1925, the list of such verified hybrids has increased so rapidly that there is little question that amphidiploidy is of general occurrence. Most of the genera which are now being critically reinvestigated by modern methods exhibit at least a few species with chromosome numbers

in multiples of some base number. Certain genera seem to have evolved exclusively by amphidiploidy, such as Chrysanthemum and Campanula.

The most striking of these recently reported hybrids are those which reproduce with the regularity of good taxonomic species. Some of them are slightly irregular, and had their progeny been further investigated would possibly have produced some different and stable derivative lines. Unfortunately the number in the latter category is small, and doubtless the behavior of all the irregular ones would vary with the degree of phylogenetic relationship. It would seem that the farther removed in relationship, the less tendency there is to the formation of quadrivalents; hence the more likelihood of breeding true from the start. Some others, however, would very probably have produced distinct derivative lines, comparable to the *N. rustica-paniculata* hybrid.

In connection with occasional irregularities, perhaps the use of pollen studies would be helpful. If it be true that the higher proportions of normal pollen grains indicate higher degrees of chromosome homology, as postulated by the writer in an earlier citation, then some notion would be gained as to the amount of disjunctional irregularity to be expected. In view of the significance of the correlation coefficients obtained between pollen percentages and estimated fertility it must be argued that pollen percentages not only indicate the degree of chromosome homology, but also the degree of fertility.

The *Crepis rubra-foetida* amphidiploid is the most irregular of those thus far described. Possibly the explanation for this fact is twofold: the close phylogenetic relation of the two parents resulting in the frequent formation of quadrivalent chromosomes in prophases, and the lower chromosome numbers of the two parents (five pairs each), in which case the gain or loss of even a single chromosome represents a greater germinal disturbance than would be the case where the parents possessed more chromosomes. At any rate, no diploid hybrid derivatives from the original cross contained a chromosome number other than 10, although 45 amphidiploid derivatives possessed approximately 1/4 with one more, and 1/4 with one less than 20 chromosomes (data not yet published).

The scarcity of aneuploids only indicates the limitations placed on the gametes in the number of chromosomes, for even within limits of 19–20–21 chromosomes the morphological variability in *rubra-foetida* was much greater than that found by LAMMERTS in his *paniculata-rustica* derivatives, where the chromosome numbers varied still more (reported verbally to the writer). Investigation of the somatic chromosomes is of importance during the first few generations, but the prohibitive size of subsequent gen-

erations places a premium on the estimation of fertility by pollen studies.

The results obtained in F_4 of the present investigation are disappointing as regards progress toward establishing stable types or increasing fertility. Yet the effort indicated some points for future investigation. The limited size of the population, about 260 plants, was due to the limited amount of cytological preparation that could be successfully undertaken, for root tips were fixed in each case. In the future much larger numbers can be raised when examination is restricted to pollen studies. Also the bagging of a few heads rather than caging of the entire plant would doubtless have avoided the evil consequences of caging, and would at the same time have permitted a truer estimate of the fertility of those plants with the highest percentage of good pollen.

In a study of sterility in pollen in Datura BLAKESLEE and CARTLEDGE (1927) present a most interesting table of aborted pollen percentages for haploids, diploids, triploids, tetraploids and trisomics. The diploid and tetraploid plants, which possess balanced sets of chromosomes, exhibit very small percentages of aborted pollen whereas the haploids and triploids exhibited 88 percent and 44 percent respectively. The primary and secondary trisomics exhibited somewhat higher proportions of abortion than the tetraploids, which is to be expected in view of the greater frequency of nondisjunction.

In the same paper they report the existence of strains of Datura which have perfectly good pollen but which when crossed regularly produce 50 percent bad pollen. Such F_1 hybrids segregate in a ratio of one-half with good pollen to one-half with 50 percent bad pollen. The explanation given is that of segmental interchange of non-homologous chromosomes in agreement with BELLING's Stizolobium crosses reported in 1914, in which the derivatives of semisterile plants continued to segregate while the completely fertile derivatives did not segregate.

BLAKESLEE and CARTLEDGE report that low temperatures directly increase the proportion of aborted pollen. However, the data from table 3 in that paper seem to be in agreement with the pollen curves obtained in the present investigation for *Crepis laciniata* and *C. mungieri*. In both the Crepis species and the Datura flowers the percentage of aborted pollen increases for several days after flowering. The Datura measurements, however, ceased after four days, whereas the Crepis measurements continued for a longer period and consequently furnish a complete history of pollen production. Therefore, the view expressed in the present investigation that temperature has no influence on pollen abortion is not contrary to BLAKESLEE and CARTLEDGE's actual figures.

It is not the intention here to emphasize the production of bad pollen through hybridization but rather the utilization of good pollen percentages for early identification of more fertile hybrid derivatives. As a result of hybridization pollen abortion may be due to: irregularities in disjunction, as shown in trisomics and hybrids with a variable tendency to form quadrivalents; segmental interchange of non-homologous chromosomes, as shown in numerous genera; and reshuffling of chromosomes of different species into incompatible reaction systems. But pollen abortion to a still more considerable extent may be due to the specific action of pollen lethal genes, as in Oenothera (SHULL 1923), and asynaptic genes, as in maize (BEADLE 1930) and Nicotiana (CLAUSEN 1931). Pollen abortion, then, may result from a number of causes, not all of which are associated with hybridization.

SUMMARY

1. Fluctuation in the percentages of good and bad pollen in pure species is probably not influenced by external factors but by the physiological adjustments made to flowering and senescence.

2. The plotted curve of good pollen grain percentages substantiates this view, indicating further that the daily fluctuation is inconsiderable in a given plant once the adjustments are made.

3. In hybrid plants differences in pollen production vary with the genetic constitution of the hybrids, the more fertile genetic constitutions possessing higher proportions of good pollen than the less fertile constitutions.

4. Inclosure of plants within cages lessens the fertility, probably due to the earlier onset of senescence.

5. Pollen studies among hybrids which are being selected for fertility permit the raising of larger generations by virtue of curtailing the required cytological examinations.

6. Pollen abortion may result either from hybridization, and consequent irregularities in disjunction, segmental interchange, and a reshuffling of chromosomes, or the specific effect of certain factors, such as pollen lethals and asynaptic genes.

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A COMPARISON OF TRIPLOID AND DIPLOID CROSSING OVER FOR CHROMOSOME II OF *DROSOPHILA MELANOGASTER*[1]

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	137
The measurement of crossing over in triploids	138
Data for chromosome II	138
Equational exceptions	144
DISCUSSION	147
CONCLUSIONS	151
LITERATURE CITED	151

INTRODUCTION

For *Drosophila melanogaster* crossing over is markedly different in triploid females and in diploid females, as has been shown by BRIDGES and ANDERSON (1925) for the X chromosome and by REDFIELD (1930) for chromosome III. Triploid crossing over may be greater or less than diploid crossing over; the sign of this difference and its absolute value depend upon the particular chromosome region involved.

Thus for the left end of the X chromosome crossing over in triploids is approximately twice that in diploids; at the right end of the X it is about half that in diploids. For the third chromosome triploid crossing over is about half diploid crossing over at either end; at the center it is more than three times as great. There is, moreover, a regular gradation in this ratio from the center of the chromosome to either end.

Regional differences had previously been found in crossing over as the result of a number of agents including age, temperature, and X-rays and it was suggested that these differences depend upon the distance of the region from the spindle-fibre attachment. The regional differences attendant upon triploidy, on the other hand, seem to be correlated with the physical spacing of the genes along the chromosome (REDFIELD 1930). It is possible that the differences caused by age, temperature, and X-rays are in the last analysis also correlated with the spacing of the genes, and

[1] Work largely completed at the MARINE BIOLOGICAL LABORATORY, Woods Hole, Massachusetts.

that these results are therefore intimately related to the data from triploids; but this can only be determined by further work.

However these questions may be answered, the triploid genetic map for chromosome III corresponds more accurately with the cytological map than does the diploid genetic map. The present paper will show that this is true also for chromosome II, a result of interest since the spatial distribution of the genes for II differs from that for III (DOBZHANSKY 1930a).

THE MEASUREMENT OF CROSSING OVER IN TRIPLOIDS

Triploid crossing over was originally measured by BRIDGES and ANDERSON (1925) by marking all three X chromosomes at the same given loci ($A_1B_1C_1 \cdots / A_2B_2C_2 \cdots / A_3B_3C_3 \cdots$). The crossing over between two given loci is then the percentage of exchange between them. This technique is well adapted to the X chromosome, for a sufficient number of sets of genes are at hand which are actually or practically allelomorphic and which can be used in the same cross without difficulties in classification. The method can be used also for the third chromosome, but with difficulty; accordingly a second method was devised and used (REDFIELD 1930). This involves the marking of only one of the three homologous chromosomes; the other two chromosomes are alike and are wild-type ($A_1B_1C_1 \cdots / A_2B_2C_2 \cdots / A_2B_2C_2 \cdots$). Between any two loci, A and B, crossing over is detected between chromosome $A_1B_1C_1 \cdots$ and either of the chromosomes of the type $A_2B_2C_2 \cdots$, but it is not detected between $A_2B_2C_2 \cdots$ and $A_2B_2C_2 \cdots$. The actual crossing over between A and B is, then, 3/2 times the apparent crossing over; this is true no matter what loci are chosen. The values obtained from such crosses are in complete agreement with those from crosses in which all three chromosomes are marked (REDFIELD 1930).

For the second chromosome a sufficient number of sets of close and classifiable genes was not available for crosses in which all three chromosomes are marked; triploid crossing over for this chromosome was of necessity studied by means of the second of the methods outlined above.

DATA FOR CHROMOSOME II

The three triploid crosses used and their diploid controls are as follows:

(A)
$$\frac{\frac{a_1^2 d_p b p_r c p_z s_p}{+}}{+} \; ♀ \times a_1^2 d_p b p_r c p_z s_p \; ♂$$

$$\frac{\frac{a_1^2 d_p b p_r c p_z s_p}{+}}{+} \; ♀ \times a_1^2 d_p b p_r c p_z s_p \; ♂$$

(B) $\dfrac{a_1{}^2 d_p b p_r}{+}$ ♀ × $a_1{}^2 d_p b p_r$ ♂

$\dfrac{a_1{}^2 d_p b p_r}{+}$ ♀ × $a_1{}^2 d_p b p_r$ ♂

(C) $\dfrac{p_r c p_z s_p}{+}$ ♀ × $p_r c p_z s_p$ ♂

$\dfrac{p_r c p_z s_p}{+}$ ♀ × $p_r c p_z s_p$ ♂.

The symbols refer to the following mutant genes: $a_1{}^2$, aristaless-2, shortens the aristae and has its locus at 0.0 on the standard diploid map; d_p, dumpy wings, at 13.0; b, black body color, at 48.5; p_r, purple eyes, at 54.5; c, curved wings, at 75.5; p_z, plexus wing venation, at 100.5; s_p, speck at the root of the wing, at 107.0.

It was originally intended that the crosses of type A would give sufficient data for crossover determinations. Good care was taken of the cultures, and parents were transferred to subcultures to minimize larval competition. But when the data were tabulated it was found that the viability of a number of classes was quite poor. Accordingly the series of mutant genes was broken into two, and crosses B and C resulted. Viability relations for these crosses were satisfactory. Curiously the crossover values for A are very near those for B and C. It follows that the inviability of the poor classes of A is largely balanced, but this could hardly be predicted without the check afforded by the later crosses.

The offspring, classified according to sex, of the three triploid crosses are shown in table 1. The percentages of the various types are much like those ordinarily obtained. There is considerable variation in the ratio of male-like to female-like individuals within the intersex class, but this is not odd for it has previously been observed (DOBZHANSKY and BRIDGES 1928) that the type of sexual development of intersexes is very susceptible to genetic modifiers. It is interesting that crosses A and B which involve the same left end of II are alike in this respect, and are unlike cross C in which a new left end of II has been substituted (and possibly also other sections outside II).

The raw data for crosses A are given in table 2. This table includes the diploid offspring—that is, all individuals which have received one representative of each chromosome from each parent. Only in these (and in the

TABLE 1

Types of offspring produced by triploid mothers.

TYPE	CROSS A		CROSS B		CROSS C		TOTAL	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
Diploid ♀	3110	51.5	3232	48.2	3328	46.3	9670	48.4
Diploid ♂	473	7.8	484	7.2	576	8.0	1533	7.7
Triploid ♀	280	4.6	386	5.8	535	7.4	1201	5.5
Intersex ♀	1095	18.1	1303	19.6	2221	30.9	4619	23.2
Intersex ♂	1060	16.7	1269	18.9	512	7.1	2841	14.2
Super ♀	1	0.2	..	..	..	..	1	0.05
Super ♂	23	3.8	22	3.3	27	3.8	72	3.6

one superfemale found) can the type of chromosome II received from the triploid mother be directly observed. Each of the other offspring has received three representatives of II, including two from the mother; what the maternal representatives are would appear only as the result of genetic tests; these tests are not feasible, for intersexes and supermales are sterile and triploids frequently do not provide enough progeny to make their composition certain.

The condensed method of presentation of the data in table 2 was suggested by Dr. C. B. BRIDGES. The "type" gives the apparent regions of crossing over, 1 representing the first region from a_1^2 to d_p, 2 the second region from d_p to b, etc. For either cross, triploid or diploid, each type contains two contrary classes; the convention followed is that the class first mentioned is always that containing a_1^2. The same method is used in table 4 and in table 6. For table 6 the leftmost mutant used is p_r; the region from p_r to c is therefore region 1, and the class first mentioned of two contrary classes is that containing p_r.

As has been stated the diploid crosses do not show for all types the expected equality of contrary classes; this is obvious, for example, in single crossovers in regions 1 and 6 and in double crossovers in 1,5 and 2,5. For the triploid crosses, of course, contrary classes are not expected to be equal; this is because the apparent type of crossover is not always the true type. The class a_1^2, for example, includes not only single crossovers in region 1 between the marked chromosome and one of the two unmarked chromosomes, but also all multiples receiving a_1^2 whose points of crossing over, after the first, are between unmarked sections.

Crossover values computed from the data of table 2 are shown in table 3; the triploid values are computed as explained briefly on page 138 and in more detail in the previous paper (REDFIELD 1930). The differences be-

TABLE 2

Diploid offspring from backcrosses of triploid females of the composition $a_1^2d_pbp_rcp_zs_p/+/+$ and of control diploid females of the composition $a_1^2d_pbp_rcp_zs_p/+$. For each type the class first mentioned is that containing a_1^2.

APPARENT TYPE	TRIPLOID CROSS	DIPLOID CROSS	APPARENT TYPE	TRIPLOID CROSS	DIPLOID CROSS
0	414+1571	529+601	1, 2, 3	0+ 0	1+ 0
1	70+ 20	95+ 24	1, 2, 4	3+ 0	1+ 1
2	179+ 125	313+276	1, 2, 5	2+ 0	5+ 0
3	75+ 56	86+ 61	1, 2, 6	1+ 0	1+ 0
4	202+ 251	228+183	1, 3, 4	0+ 0	4+ 0
5	100+ 114	266+238	1, 3, 5	0+ 0	2+ 1
6	16+ 57	31+ 60	1, 4, 5	0+ 0	6+ 0
1, 2	6+ 5	10+ 3	1, 4, 6	0+ 0	4+ 1
1, 3	9+ 4	8+ 5	2, 3, 4	2+15	4+ 4
1, 4	14+ 13	28+ 11	2, 3, 5	1+ 2	6+15
1, 5	4+ 7	31+ 3	2, 3, 6	0+ 4	0+ 1
1, 6	2+ 0	11+ 1	2, 4, 5	0+ 3	7+19
2, 3	1+ 13	0+ 11	2, 4, 6	2+ 2	0+ 4
2, 4	12+ 49	22+ 98	2, 5, 6	0+ 2	0+ 1
2, 5	3+ 18	17+120	3, 4, 5	0+ 1	3+ 0
2, 6	5+ 8	51+ 27	3, 4, 6	0+ 0	2+ 4
3, 4	7+ 17	58+ 22	4, 5, 6	1+ 0	3+ 0
3, 5	3+ 5	14+ 22	1, 2, 3, 4	0+ 0	2+ 0
3, 6	9+ 5	79+ 7	1, 2, 3, 5	0+ 0	1+ 0
4, 5	2+ 36	4+ 25	1, 2, 4, 5	0+ 0	3+ 0
4, 6	13+ 14	18+ 10	..	..	..
5, 6	3+ 3	2+ 4	N	3583	3819

tween the triploid values and the diploid values are obvious. For region 1, from aristaless to dumpy, triploid and diploid crossing over are almost the same. For the second region from dumpy to black triploid crossing over is less than diploid crossing over, the ratio of the two being 0.72. From this region to the center of the chromosome, triploid crossing over increases relative to diploid crossing over, until for the section from purple to curved the triploid value is 1.35 times the diploid value. The next section to the right, namely from curved to plexus, again shows a decrease in triploid crossing over, the quotient falling to 0.61. At the right end of the chromosome from plexus to speck the ratio rises reaching a value of 0.74. Thus the second chromosome can be divided at the center into two limbs which behave similarly with regard to the relation of triploid crossing over to diploid crossing over. A like division into two limbs of chromosome III for triploids has previously been found (REDFIELD 1930). There are differences between the limbs of chromosome II and chromosome III and these differ-

ences, as will be shown, are correlated with corresponding differences in the spacing of the genes in these chromosomes.

It may be noted that the total crossing over from aristaless to speck for chromosome II is somewhat less (about 13 units less) in the triploid than in the diploid. In the crosses for chromosome III which involved the entire distance from roughoid to claret the total triploid crossing over was also appreciably less than the total diploid crossing over (REDFIELD 1930). Thus it would appear that the total frequency of crossing over is not necessarily the same in the triploid and in the diploid.

TABLE 3

Crossover values (with probable errors) computed from the data of table 2.

REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T−D	QUOTIENT T/D
$a_1^2-d_p$	6.7±0.28	6.9±0.28	−0.2±0.39	0.97±0.06
d_p-b	19.4±0.45	26.8±0.48	−7.4±0.66	0.72±0.02
$b-p_r$	9.6±0.33	11.3±0.35	−1.7±0.48	0.85±0.04
p_r-c	27.6±0.50	20.4±0.44	+7.2±0.67	1.35±0.04
$c-p_z$	13.0±0.38	21.4±0.45	−8.4±0.59	0.61±0.02
p_z-s_p	6.2±0.27	8.4±0.30	−2.2±0.41	0.74±0.04
Total	82.5±0.93	95.2±0.96	−12.7±1.33	..

The data for crosses of type B are given in table 4 and the corresponding crossover values in table 5. The ratios of triploid to diploid crossing over are somewhat different than those for crosses of type A, but they are not more different than might be expected, and the change in the ratio along the chromosome is of the same nature as for crosses A. Thus the ratio is greater for $a_1^2-d_p$ than for d_p-b, and increases again for the region $b-p_r$ near the center of the chromosome.

TABLE 4

Diploid offspring from backcrosses of triploid females of the composition $a_1^2 d_p b p_r/+/+$ and of control diploid females of the composition $a_1^2 d_p b p_r/+$. For each type the class first mentioned is that containing a_1^2.

APPARENT TYPE	TRIPLOID CROSS	DIPLOID CROSS	APPARENT TYPE	TRIPLOID CROSS	DIPLOID CROSS
0	780+2199	2500+2874	1, 2	9+12	20+14
1	93+ 82	420+ 422	1, 3	6+ 4	25+15
2	192+ 166	1143+1273	2, 3	8+14	17+32
3	75+ 76	234+ 201	N	3716	9190

Similar data and crossover values for crosses of type *C* are given in tables 6 and 7. For the chromosome limb covered by these crosses, namely

TABLE 5

Crossover values (with probable errors) computed from the data of table 4.

REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T−D	QUOTIENT T/D
$a_1^2-d_p$	8.3±0.31	10.0±0.21	−1.7±0.37	0.83±0.04
d_p-b	16.2±0.41	27.2±0.32	−11.0±0.52	0.59±0.02
$b-p_r$	7.4±0.29	5.7±0.16	+1.7±0.33	1.30±0.06

the right limb, the values of the ratio are practically the same as for crosses *A*. The quotient decreases as we pass from the end of the chromosome to intermediate regions; it increases as we pass from these intermediate regions to the center of the chromosome where it reaches a value of about 1.4.

TABLE 6

Diploid offspring from backcrosses of triploid females of the composition $p_rcp_xs_p/+/+$ and of control diploid females of the composition $p_rcp_xs_p/+$. For each type the class first mentioned is that containing p_r.

APPARENT TYPE	TRIPLOID CROSS	DIPLOID CROSS	APPARENT TYPE	TRIPLOID CROSS	DIPLOID CROSS
0	707+1948	2481+2871	1,3	7+12	33+38
1	338+ 288	797+ 797	2,3	4+ 0	1+ 4
2	129+ 147	993+ 946	1,2,3	1+ 0	3+ 0
3	39+ 51	203+ 261	..	..	..
1,2	27+ 32	97+ 78	N	3904	9603

TABLE 7

Crossover values (with probable errors) computed from the data of table 6.

REGION	TRIPLOID T	DIPLOID D	DIFFERENCE T−D	QUOTIENT T/D
p_r-c	27.1±0.48	19.2±0.27	+7.9±0.55	1.41±0.02
$c-p_x$	13.0±0.36	22.1±0.29	−9.1±0.46	0.59±0.02
p_x-s_p	4.4±0.22	5.7±0.20	−1.3±0.30	0.77±0.06

The results of crosses *B* and *C* are shown graphically in figure 1; results from crosses *A* are not included since poorer viability relations make them less certain. The figure shows the relatively low value of the ratio at intermediate regions, its slight increase at the ends of the chromosome, and its definite and greater increase at the center of the chromosome. It should be noted that three of the regions concerned represent relatively long crossover sections, namely d_p-b, p_r-c, and $c-p_x$. For these regions a small

correction for undetected doubles would result if it had been possible to deal with shorter sections. In this case the general shape of the curve would have remained the same, but it would undoubtedly have shown minor changes.

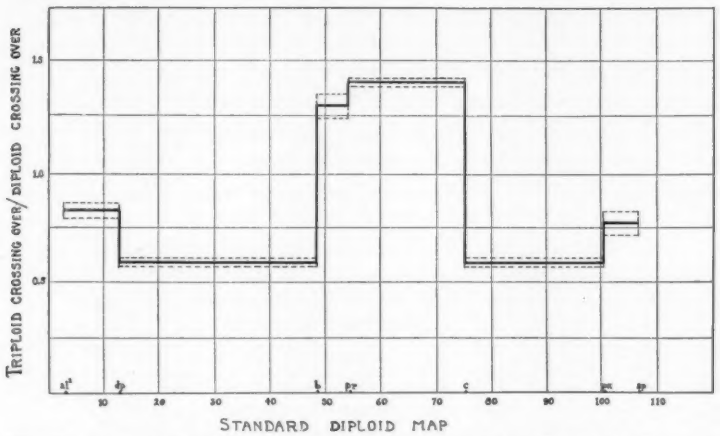

FIGURE 1.—The relation between triploid and diploid crossing over along the chromosome. Ordinates represent the ratio of triploid crossing over to diploid crossing over, abscissae the arrangement of the genes as shown by the standard diploid map. Dotted lines give the probable errors for the respective regions.

EQUATIONAL EXCEPTIONS

Triploid females containing a single dose of a given mutant gene produce occasional individuals which have received two maternal representatives of this gene. Since the diploid father contributes one chromosome II, such equational exceptions for II are found only among those types which have arisen from diploid eggs and which have three representatives of the locus in question, namely among the triploids, intersexes, and supermales.

If in the formation of the diploid eggs chance alone determined which two of six allelomorphic genes passed to a given pole, 1/15 or 6.66 percent of these eggs would contain two sister mutant genes. In other words, 6.66 percent of the triploids, intersexes, and supermales would in the present crosses show aristaless, or dumpy, or any other of the recessive mutant characters used. But it has been shown for the X chromosome of triploids (BRIDGES and ANDERSON 1925) and for chromosome III of triploids (REDFIELD 1930) that sister genes normally separate at the spindle-fibre

attachment, and that the tendency to separate becomes less the farther the locus is from the spindle fibre; the association does not become random, however, even for those genes which are farthest from the spindle-fibre attachment. Equational exceptions depend therefore upon crossing over between the locus and the spindle-fibre attachment resulting in an association of sister genes which would otherwise have been separated.

Thus the tendency in triploids of sister genes to separate may be used to place the spindle-fibre attachment. In this manner the attachment to the X has been shown to be at the right end of the chromosome, and to III at the center of the chromosome. These results are in agreement with previous determinations from studies of equational exceptions produced by attached-X diploids (ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT 1931), from equational exceptions produced by a high non-disjunction line (ANDERSON 1929), and from studies of translocations (STERN 1926, MULLER and PAINTER 1929, DOBZHANSKY 1930b).

DOBZHANSKY'S examination (1930a) of translocations involving the second chromosome has shown that the spindle fibre is attached at the center of II slightly to the right of purple. This had long been suspected (BRIDGES and MORGAN 1923) and is in agreement with the results of the present paper.

Equational exceptions from triploid females of the composition $a_1^2\ d_p\ b\ p_r\ c\ p_z\ s_p/+/+$ are shown in table 8. The classification of curved in intersexes is not certain for intersexes often have their wings spread apart, but it was thought it would do no harm to attempt it, and the results of

TABLE 8

Equational exceptions from triploid females of the composition $a_1^2 d_p b p_r c p_z s_p/+/+$.

CLASS	TRIPLOID	♀ INTERSEX	♂ INTERSEX
a_1^2	1	6	9
$a_1^2 d_p$	..	4	2
$a_1^2 d_p b$	..	2	..
$a_1^2 c p_z s_p$	..	1	..
d_p	..	..	1
$b p_r c p_z s_p$	..	1	..
$b p_r p_z s_p$	..	..	1
$b c p_z s_p$	..	1	1
$b s_p$	..	1	..
p_r	..	1	..
$c p_z s_p$	..	12	5
$c p_z$	1	..	..
$p_z s_p$	4	8	4
s_p	2	3	6

table 11 show that it was not far wrong. Table 9 gives the equational exceptions for $a_1^2\ d_p\ b\ p_r/+/+$ triploids, and table 10 for $p_r\ c\ p_x\ s_p/+/+$ triploids. In the intersexes of the latter cross the classification of curved was very difficult and was omitted.

TABLE 9

Equational exceptions from triploid females of the composition $a_1^2 d_p b p_r/+/+$.

CLASS	TRIPLOID	♀ INTERSEX	♂ INTERSEX
a_1^2	5	3	3
$a_1^2 d_p$	..	5	7
$a_1^2 d_p b$	..	2	..
$a_1^2 d_p b p_r$	1	2	4
d_p	..	1	2
$d_p b$	..	1	..
$d_p b p_r$	..	1	..
b	2	1	..
$b p_r$	..	2	..
p_r	..	1	..

TABLE 10

Equational exceptions from triploid females of the composition $p_r c p_x s_p/+/+$. Curved could not be classified in the intersexes of these crosses and is therefore omitted.

CLASS	TRIPLOID	♀ INTERSEX	♂ INTERSEX
p_r	1	4	1
$p_r p_x s_p$	..	1	..
p_x	2	3	1
$p_x s_p$	6	32	9
s_p	3	5	..

Table 11 shows the numbers and percentages of representation of each gene in the equational exceptions for the three types of cross. In each case

TABLE 11

Representations of the genes in the equational exceptions. Chance expectation is 6.66 percent.

	CROSS A		CROSS B		CROSS C	
GENE	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
a_1^2	25	1.03	32	1.08	..	..
d_p	9	0.37	26	0.88	..	..
b	7	0.29	16	0.54	..	..
p_r	3	0.12	11	0.37	7	0.21
c	22	0.91	..	..	..	..
p_x	39	1.60	..	..	54	1.65
s_p	50	2.05	..	..	56	1.71

there is a regular gradation in the percentage of equational exceptions from the low value at the center of the chromosome to the relatively high value at either end. The lowest value reached for any of the loci is for purple, which means that the spindle-fibre attachment is nearest purple.

An examination of the classes of tables 8, 9, and 10 shows, moreover, that this attachment must be somewhat to the right of purple between purple and curved, rather than between black and purple. This is because purple tends to appear with genes to its left and not with those to its right. There are exceptions to this tendency, namely the $b\ p_r\ c\ p_z\ s_p$ female intersex and the $b\ p_r\ p_z\ s_p$ male intersex of table 8, and the $p_r\ p_z\ s_p$ female intersex of table 10. These, no doubt, are due to double crossing over, one point of crossing over being to the left of the spindle fibre but to the right of purple, the other to the right of the spindle fibre and either to the left or right of curved.

In no case does the percentage of representation approach that expected on the basis of random assortment (6.66 percent). In this respect the data are like those obtained for the X chromosome of triploids (BRIDGES and ANDERSON 1925) and for chromosome III of triploids (REDFIELD 1930). This may be due partially to low viability, but there is at least one other factor concerned. This is the fact that of the diploid eggs only those are available for equational exceptions which contain at least one chromosome representing recombination between the locus and the spindle-fibre attachment. It is of course an easy matter to calculate an approximation to the expected percentage of recombination between the spindle-fibre region and aristaless or speck near either end of the chromosome. When this is done the expected percentages of equational exceptions for the ends of the chromosome are higher than, but are not far from, the percentages actually obtained. However, these matters are undoubtedly further complicated by additional factors about which nothing is known so it seems advisable to postpone discussion.

DISCUSSION

Polyploid crossing over is not always markedly different from diploid crossing over. In the Drosophila triploid, as has been shown, there are regions for which the crossover values are the same as those in the diploid. The recent papers of SVERDRUP SÖMME (1930) and of DE WINTON and HALDANE (1931) have shown that crossover values are not far different in the tetraploid and in the diploid forms of *Primula sinensis*. It is, of course, possible that chromosome regions other than those with which they were able to deal will be found to give differing values. On the other hand, the

situation in Primula is somewhat different from that in Drosophila. So far as the number of strands involved is concerned, the tetraploid Primula may be regarded as a modified diploid type; for it is probable, as is pointed out by DE WINTON and HALDANE, that crossing over in these tetraploids involves only two of the four homologous chromosomes. In Drosophila it seems probable (ANDERSON 1925) that only two strands cross over at any one level, but the equality of recurrent and progressive double crossovers (BRIDGES and ANDERSON 1925, REDFIELD 1930) demonstrates that the chromosomes of the triploid of this form conjugate by threes and that the strands taking part in a first crossing over do not determine which strands take part in a second.

However it is well not to overemphasize this distinction. It would seem that a substitution of six sets of crossover strands in the triploid Drosophila (BRIDGES and ANDERSON 1925) for the four sets present in the diploid might in itself give rise to differences in crossing over, in particular as the result of sister-strand crossing over. Still there is evidence from the use of crossover reducers (REDFIELD unpublished) that when four strands alone of the six are allowed to cross over in the triploid cell the values obtained are triploid and not diploid. DOBZHANSKY has shown (1929a) that triploid cells in Drosophila differ from diploid cells not only in the number of crossing over strands but also in their general size relationships. It is conceivable that marked alterations in the relative size of the cell components might result in differences in crossing over.

From other considerations also it may be concluded that sister-strand crossing over cannot alone be held accountable for the differences in crossover values between triploid and diploid. If crossing over occurred between the two sister strands resulting from the splitting of a single chromosome its results would not be directly detected in either triploid or diploid. But if it occurred freely the net crossover values for the triploid would show a definite increase over the values for the diploid. For in this case the diploid values obtained would represent 2/3 the actual physical crossing over whereas the triploid values would represent 4/5 the actual total crossing over. It is clear, however, that the regional differences along the chromosome for the X, II, and III completely mask any constant difference due to sister-strand crossing over. Indeed, triploid crossing over may be considerably lower than diploid crossing over (the right end of the X, intermediate regions in II, and the ends of III). Thus the possibility of sister-strand interchange cannot be directly investigated by a comparison of triploid and diploid values, but it is believed it can be investigated, although with difficulty, by the use of crossover reducers in triploids; such crosses are now well under way.

Whatever may be the decision as to sister strand interchange (the very slight evidence we now have indicates that it does not occur, STURTEVANT 1928), the regional differences between triploid and diploid crossing over will still remain unexplained. And it is exactly these regional differences which are most striking in comparing diploid and triploid.

In an examination of the differences the first relation which occurs to one is the curious fact that chromosome regions in which crossing over in the triploid is increased are regions in which genes are clumped on the standard diploid maps. The converse statement also holds: regions in which crossing over in triploids is markedly decreased are regions in which genes are sparse on the diploid genetic maps. The former case is illustrated by the left end of the X, the center of II, and the center of III; the latter by the right end of the X, intermediate regions of II, and the ends of III. Thus there is a definite relationship between the spacing of the genes on the regular diploid maps and the differences between triploid and diploid crossing over.

Now it had long been suspected that there are differences between the spacing of the genes on the maps and their actual physical spacing along the chromosomes (BRIDGES and MORGAN 1923, MORGAN, BRIDGES, and STURTEVANT 1925). The studies of crossover reducers led to the suspicion that regions in which genes are clumped on the maps are regions in which crossing over is relatively lower per unit of physical distance between genes, that is, that the unit of crossing over varied from region to region. In most cases, then, the clumping of the genes was supposed to represent a genetic phenomenon rather than a physical condensation of genes.

The recent results from translocations (largely made possible by the introduction of X-rays into genetic studies) showed that this is indeed true. The first direct evidence of this type was presented by MULLER and PAINTER in a general preliminary paper (1929) reporting a number of different types of translocation. There followed the detailed studies of DOBZHANSKY (1929b, 1930b) on translocations involving the third and fourth chromosomes. He found on direct observation of the metaphase plates of these translocations and on genetic analysis of the points of breakage that for chromosome III "the distances between the loci of the breakages or of the genes lying in the middle part of the chromosome are longer cytologically than they might be expected to be on the basis of the genetical map. On the other hand, the distances between the loci lying near the ends of the chromosome are shorter cytologically than they might be expected to be on the basis of the genetical data. The genetical map represents the genes located in the middle part of the chromosome (near

the spindle fibre) relatively too close to each other, and the genes located at the ends of the chromosome relatively too far apart."

These results correspond well with those found by REDFIELD (1930) in a comparison of crossing over in the triploid and diploid Drosophila for the third chromosome. "Crossing over at the ends of the chromosome is slightly more than one-half as great in the triploid as in the diploid; and as we pass from either end of the chromosome to the center the relative amount of crossing over in the triploid increases continuously until we reach a maximum at the center, for which region triploid crossing over is more than three times as great as diploid crossing over."

A subsequent study of translocations involving the second chromosome (1930a) led DOBZHANSKY to conclude for II: "The distances between the genes located at the middle of the chromosome are much longer cytologically than suggested by the genetic map. On the contrary, distances between the genes located to the right and to the left of the middle of the chromosome are relatively far shorter cytologically than the genetic map indicates. Finally, the distances between the genes located near the right end of the chromosome are again relatively longer cytologically than indicated by the genetic map. It is probable that the same is true in respect to the genes located at the extreme left end of the chromosome, though it is not certain."

It is immediately obvious that the present paper shows results of the same general nature. Triploid crossing over is about 1.4 times as great as diploid crossing over for central regions of II. The ratio decreases to about 0.6 for intermediate regions to the right and left of the central regions; it increases again for the terminal regions at either end of this chromosome although, curiously enough, it does not reach unity.

It should be emphasized that these statements are not intended to imply that triploid crossover values are directly proportional to distances on the cytological maps; it would be very surprising if such a simple relationship did hold, and indeed we are certain it does not. If it did we should expect, for example, that triploid values for the right end of II would be relatively much greater than they actually are.

It cannot yet be stated with certainty that the X chromosome fits into this scheme, although it would seem that the data suggest that it does. It has long been known that there is a definite clumping of genes at the left end of this chromosome and it has consequently been supposed that the left end is longer relative to the rest of the chromosome than the genetic map indicates. This fits very well with the results of BRIDGES and ANDERSON (1925) for the X chromosome of triploids which showed that crossing

over at the left end in triploids is approximately twice that in diploids, and that for the rest of the chromosome it is about half that in diploids. Other unpublished results (REDFIELD) give differences in the same direction and even more extreme for the ends of the chromosome and with a regular gradation from one end to the other.

But from a preliminary note published by PAINTER (1931) it would appear that these relations do not agree with his cytological map. Until this cytological question is definitely settled for the X, it is perhaps best not to discuss the matter further.

CONCLUSIONS

(1) For central regions of the second chromosome of *Drosophila melanogaster* triploid crossing over is greater than diploid crossing over (T/D is about 1.4); for the regions to the right and to the left of the center it it much less than diploid crossing over (T/D is about 0.6); and for regions at either end of the chromosome triploid crossing over again increases relative to diploid crossing over but does not become equal to it.

(2) The total crossing over from aristaless to speck is about 13 units less in the triploid than in the diploid.

(3) Triploid crossing over tends to increase relative to diploid crossing over in regions which are longer cytologically than the chromosome map indicates and tends to decrease relative to diploid crossing over in regions which are shorter cytologically than the chromosome map indicates. This conclusion is based upon a comparison of the present results with the cytological map of DOBZHANSKY for chromosome II. It is not implied that triploid crossover values are directly proportional to distances on the cytological maps.

(4) Triploid females containing a single dose of a given mutant gene produce occasional equational exceptions which have two maternal representatives of this mutant gene. The percentage of equational exceptions shows a regular gradation from the low value for genes at the center of chromosome II to the relatively high value for genes at either end. In no case does this percentage approach that expected on the basis of random assortment.

(5) These equational exceptions provide new and independent evidence that the spindle-fibre attachment is at the center of chromosome II slightly to the right of purple. This is in agreement with DOBZHANSKY'S previous evidence from a study of translocations.

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THE BRISTLES OF HYBRIDS BETWEEN *DROSOPHILA MELANOGASTER* AND *DROSOPHILA SIMULANS**

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	153
Material and methods	153
Success of crosses	156
Preliminary crosses	158
Crosses to test cytoplasmic differences in *melanogaster* stocks	160
The use of *melanogaster* attached-X stocks	162
Effect of *melanogaster* cytoplasm	165
Influence of *simulans* stocks	167
Bristles of the parent species	170
DISCUSSION OF RESULTS	171
SUMMARY	172
LITERATURE CITED	173

INTRODUCTION

In 1920, STURTEVANT found that hybrids from *Drosophila melanogaster* by *D. simulans* have certain bristles missing from the body that are normally present in both parent species. He discovered later (1929) that the number of bristles present (counting dorsocentrals) in these hybrids varies with the *melanogaster* stock used, and that the *simulans* male apparently produces no effect on bristle number. This suggested that the *melanogaster* egg-cytoplasm might cause the effect, but a preliminary test gave negative results.

The present paper contains the results of a detailed study of the inheritance of the differences among the stocks of the pure species, with respect to their effects on the bristles of the hybrids.

I wish to express my appreciation to Professor A. H. STURTEVANT for his direction of the work, and to Professor T. H. MORGAN and Professor TH. DOBZHANSKY for their helpful suggestions. The research was carried on at the CALIFORNIA INSTITUTE OF TECHNOLOGY, Pasadena, California.

MATERIAL AND METHODS

The various *melanogaster* stocks which were used are listed alphabetically in table 1. Stocks inbred for many generations were used, when possible, to insure a condition homozygous for any modifiers present. An at-

* Part of the cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

TABLE 1

List of D. melanogaster stocks used in the experiments.

SYMBOL	MEANING OF SYMBOL	CHROMOSOME
$\frac{b\ g_p\ a}{C_y R}$	Black gap arc over the crossover-suppressor $d_p^2\ C_y C_{IILC_y}\ p_r C_{IIR}{}^l\ p_x\ s_p$	II
$\frac{B_l}{C_y\ s_p}$	Bristle[1] over the crossover-suppressor $C_y\ C_{IILC_y}\ c_n{}^2\ C_{IIRC_y}\ s_p$	II
$\frac{c_g}{d_p{}^2 C_y}$	Comb-gap[2] over the crossover-suppressor $d_p{}^2\ C_y\ C_{IILC_y}\ c_n{}^2\ C_{IIRC_y}$	II
$\widehat{ff}\ b\ p_r\ c\ s_e\ h$	Stock containing attached X chromosomes bearing forked, and homozygous for black purple curved and sepia hairy	I, II, III
$\frac{f_r\ s_p}{C_y}$	Fringed speck over the crossover-suppressor $C_y\ C_{IILC_y}\ c_n{}^2\ C_{IIRC_y}$	II
$\frac{f_u}{ClB}$	Fused over the crossover-suppressor $s_c\ f^2\ v\ C_I\ l\ s_l\ B$	I
$\frac{g_l\ bb''}{ClB}$	Giant[2] bobbed—11 over the crossover-suppressor *ClB*	I
$\frac{l_z}{ClB}$	Lozenge over the crossover-suppressor *ClB*	I
$\frac{p_n{}^2}{\widehat{yy}}$	Stock containing prune—2 males and yellow females with attached X's	I
$\frac{p_x\ b_w\ m_r\ s_p}{C_y}$	Plexus brown morula speck over the crossover-suppressor $C_y\ C_{IILC_y}\ c_n{}^2\ C_{IIRC_y}$	II
res	Homozygous stock: roughoid hairy thread scarlet peach curled stripe sooty	III
$\frac{res}{C_{FL}C_{FR}}$	"*res*" over a crossover-suppressor[5]	III
$\frac{res}{D_f\ 2C\ c_a}$	"*res*" over the crossover-suppressor $l\ C_{IIILP}\ D_f\ l\ C_{IIIRP}\ c_a{}^3$	III
rucuca	Homozygous stock: roughoid hairy thread scarlet curled stripe sooty claret	III
$\frac{s^2 f}{ClB}$	Sable—2 forked over the crossover-suppressor *ClB*	I
$s_e\ h$	Homozygous stock: sepia hairy. There was also a stock $\frac{s_e\ h}{C_{FL}\ C_{FR}}$	III
$s_l\ s_r\ e^4\ r_o\ c_a$	Homozygous stock: scarlet stripe ebony—4 rough claret. There was also a stock $\frac{s_l\ s_r\ e^4\ r_o\ c_a}{C_{FL}\ C_{FR}}$	III
$\frac{s_l\ s_r\ e^4\ r_o\ c_a}{C_{IIILP}C_{IIIRP}{}^l}$	Scarlet stripe ebony—4 rough claret over a crossover-suppressor	III
$\frac{l_a}{\widehat{yy}}$	Stock containing twisted-abdomen males and yellow females with attached X's	I

TABLE 1 (continued)

SYMBOL	MEANING OF SYMBOL	CHROMOSOME
thes	Homozygous stock: thread scarlet peach curled stripe sooty. There were also stocks $\frac{thes}{C_{FL}C_{FR}}$ and $\frac{thes}{C_{IIILP}C_{IIIRP}l}$	III
$\frac{v\,g^2\,s_1 f}{ClB}$	Vermilion garnet—3 small-wing forked over the crossover-suppressor *ClB*	I
$\frac{y\,U\,c_v\,v\,f}{s_c\,c_v\,v\,f}$	Stock producing females with U-shaped X chromosome containing yellow crossveinless vermilion forked over scute crossveinless vermilion forked[4]	I
$\frac{y\,U\,c_v\,v\,f}{s_c\,e_c\,c_t^8\,g^2}$	Stock producing females with $y\,U\,c_v\,v\,f$ overs cute echinus cut—6 garnet—2	I
$\frac{y\,U\,e_c\,c_t^8\,g^2}{s_c\quad e_c\,c_t^8\,g^2}$	Stock producing females with $y\,U\,e_c\,c_t^8\,g^2$ over $s_c\,e_c\,c_t^8\,g^2$	I

For a more complete description of the characters see MORGAN, BRIDGES and STURTEVANT 1925.

[1] For Bristle, see KING 1927.

[2] For giant, see MORGAN, STURTEVANT and BRIDGES 1927.

[3] For $D_f 2Cc_a$, see MORGAN, STURTEVANT and BRIDGES 1928.

[4] For *yU*, see L. V. MORGAN 1926.

[5] No published data for c_g or $C_{FL}C_{FR}$.

tempt was made to keep in touch with the whole chromosome, as far as possible, by using crossover suppressors preventing the loss of modifying genes through crossing over in the female parent. All the *simulans* stocks had been inbred for many generations. (For a more complete description of all the stocks, except Pasadena, see STURTEVANT 1929. There are no published data for the Pasadena stock.) The stocks of wild-type or normal flies are named after the towns in which the flies were collected. They are Morristown (Mrstwn.) from Morristown, New Jersey, New Orleans (N. Orl.) from New Orleans, Louisiana, Pasadena (Pas.) from Pasadena, California, and St. Augustine (St. Aug.) from St. Augustine, Florida. The first chromosome stocks include homozygous yellow prune ($y\,p_n$), yellow white ($y\,w$), and a homozygous yellow stock (Metz Fla. y) collected by C. W. METZ in Florida. There is also homozygous black (b) in the second chromosome and homozygous sepia (s_e), scarlet (s_t), and scarlet peach ($s_t\,p$) in the third chromosome.

In making the crosses small mass-cultures were used, consisting of 4 or 5 *melanogaster* females with 8 to 10 *simulans* males, or 8 to 10 *simulans* females with 4 or 5 *melanogaster* males. When the combination was made in this way the cross of *simulans* ♀ by *melanogaster* ♂ caused less difficulty than had been experienced previously by STURTEVANT, MORGAN, BONNIER and others. The more frequent failure of the latter combination

is due to the lower viability of *simulans* flies in wet and acid food conditions. The females may become weakened and die before laying many eggs or even before mating. To overcome this difficulty the flies were mated and kept in vials with a small amount of food for one day before transferring them to the culture bottles. The bottles were kept in an incubator set at 25.5° C until pupae had formed. The parents were then removed and the bottles kept at room temperature (20–22° C).

The chance that non-virgin females may be present increases with the number of females used in a mating. Since a large number of *simulans* females were used in each cross greater care was required in selecting virgins. There was the added difficulty that *simulans* females are sometimes fertilized a few hours after emerging (MORGAN 1929). Hybrid cultures generally consist of only one sex (see STURTEVANT 1920). This served as a check, but in addition all the crosses were made in such a way that flies which were not hybrids could be detected immediately by other characters.

In counting the bristles the four dorsocentrals and four scutellars were considered. To save time in making and recording the counts it was decided at first to include flies with more than four of the eight bristles missing in the class of "four missing." This tended to lower the computed mean number of missing bristles in cases where many bristles were absent, but did not affect the cases where a smaller number were absent. If only four of the eight bristles had been considered the low classes would have been reduced by approximately half and the differences between them would hardly be apparent. The differences between the various high classes are sufficient, as recorded, to distinguish them from one another. Recording all eight bristles would make the differences greater instead of less. In the later counts all eight bristles were recorded.

SUCCESS OF CROSSES

It is easier to make the cross using *melanogaster* females than *simulans* females. This had been found previously by STURTEVANT, MORGAN and others, but the difficulty with *simulans* females was not as great as in their experiments. This may have been due to the methods that were used. STURTEVANT (1929) and MORGAN (1929) expressed the opinion that the yellow stocks gave a greater percent of successful crosses since the females were less vigorous and offered less opposition to mating. In the present experiment the yellow *simulans* stocks gave poor results and the yellow *melanogaster* stocks gave about the average number of successful matings.

In a few cases crosses with *melanogaster* females were one hundred per-

cent successful out of five or six matings, but in some other cases as many as fifty unsuccessful attempts at crossing were made. The figure for the success of all crosses with *melanogaster* females, 44 percent, is really not a true index since an equal number of attempts at each cross was not made. The values range from 0 percent to 86 percent for the *simulans* males used and from 0 percent to 100 percent for the *melanogaster* females. The stock which gave 100 percent included only six cultures; the next highest value is 68 percent.

The values for successful crosses with *simulans* females range from 8 percent to 100 percent for the *simulans* females and from 0 percent to 83 percent for the *melanogaster* males. The figure for all crosses, 45 percent, is again not a true index as to the success of the matings. It must be remembered that an average of ten *simulans* females were used for each culture while only five *melanogaster* females were used in the crosses.

Considering all the matings the black *simulans* stock was found to give the highest percent of successful crosses, 79 percent. The New Orleans stock came next with 68 percent. In the *melanogaster* stocks $\widehat{yy}$ C_y from (F) was first with 70 percent and $\frac{C_y}{+}\ \frac{s_a\ h}{+}$ from (B) second with 67 percent of successful crosses.

A summary of most of the crosses with the mean number of bristles missing is given in table 2.

TABLE 2

Mean number of bristles missing in hybrids between Drosophila melanogaster and D. simulans.

simulans STOCK ♂ AND ♀	*melanogaster* STOCK											
	$\frac{C_y\ s_a h}{+\ +\ }$♀ FROM (A) AND (B)		$\frac{C_y\ s_a h}{+\ +\ }$♂ FROM (A) AND (B)		$\frac{C_y\ s_a h}{\text{II}\ +}$♀ FROM (D)		$\frac{C_y\ s_a h}{\text{II}\ +}$♂ FROM (D)		$\frac{C_y\ s_a h}{\widehat{yy}\ \ Df}$♀ FROM (C)		$\frac{\widehat{yy}\ ♀ \text{ FROM } p_n^2}{\widehat{yy}}$	$\frac{\widehat{yy}\ ♀ \text{ FROM } t_a}{\widehat{yy}}$
	C_y ♀ +		C_y ♂ +		C_y ♀ +		C_y ♂ +		C_y ♂ +		♂	♂
s_a	0.23	0.18	♂1.50 ♀0.55	.52 .21	0.27	0.15	2.06	0.62	3.04	1.44	2.28	1.83
N. Orl.	..	..	..	..	0.59	0.23	1.84	0.29	2.85	1.38	2.26	1.93
Pas.	..	..	..	..	0.19	0.07	1.60	0.41	3.04	1.55	1.81	1.89
Mrstwn.	0.40	0.28	1.25	0.13	0.85	0.45	1.20	0.15	1.51	0.54	0.78	0.74
St. Aug.	0.36	0.33	..	..	..	..	3.36	1.32	..	..	2.19	1.32
b	..	..	..	..	1.26	0.46	2.70	0.79	..	..	2.92	2.73
yw	..	..	..	..	..	..	..	..	..	..	..	2.53
yp_n	..	..	..	..	..	..	..	..	..	..	..	1.38

TABLE 3

Hybrids produced by all crosses.

TYPE OF CROSS	FEMALE HYBRIDS	MALE HYBRIDS	TOTALS
mel. ♀ × *sim.* ♂	20,388	41	20,429
$\widehat{XXY}$ *mel.* ♀ × *sim.* ♂	3	15,045	15,048
sim. ♀ × *mel.* ♂	437	16,870	17,307
Totals	20,828	31,956	52,784

Table 3 shows the number of hybrids produced by all the crosses. The 41 males produced by regular *melanogaster* females were due to non-disjunction in females containing *ClB*. The 3 females produced by attached-X *melanogaster* females were due to separation of the attached X's in the mother.

PRELIMINARY CROSSES

It was first necessary to find various *melanogaster* stocks that produced different bristle effects on the hybrids. Females from stocks containing genes in the three large linkage groups were crossed to males from various *simulans* stocks. In the case of the X and the second chromosome it was possible to use stocks containing recessive genes as well as a dominant gene with linked crossover suppressors, such as $\frac{g_1\, bb''}{ClB}$ or $\frac{b\, g_2\, a}{C_1 R}$. Thus in the

TABLE 4

Preliminary crosses involving chromosome I.

melanogaster ♀	*simulans* ♂	NUMBER OF FLIES	MEAN NUMBER OF BRISTLES MISSING	
			B	+
$\frac{f_{36}}{ClB}$	N. Orl.	125	1.09	0.85
$\frac{g_1 bb''}{ClB}$	Mrstwn.	31	0.00	0.52
$\frac{g_1 bb''}{ClB}$	St. Aug.	361	2.11	2.47
$\frac{g_1 bb''}{ClB}$	s_8	284	0.31	0.47
$\frac{l_2^4}{ClB}$	N. Orl.	838	1.02	0.73
$\frac{l_2^4}{ClB}$	St. Aug.	40	2.72	2.68
$\frac{s^2 f}{ClB}$	s_8	282	0.25	0.18

hybrids the effect on the bristles of each of the homologous chromosomes could be determined by comparing the normal hybrids with those showing the character due to the dominant gene. There was no suitable third

TABLE 5

Preliminary crosses involving chromosome II.

melanogaster ♀	*simulans* ♂	NUMBER OF FLIES	MEAN NUMBER OF BRISTLES MISSING	
			C_y	+
$\frac{b\ g_p\ a}{C_y\ R}$	Mrstwn.	58	1.00	0.80
$\frac{b\ g_p\ a}{C_y\ R}$	N. Orl.	460	1.82	1.06
$\frac{c_g}{d_p^2\ C_y}$	N. Orl.	394	0.73	0.99
$\frac{f_r\ s_p}{C_y}$	Mrstwn.	433	0.65	1.82
$\frac{p_z\ b_\omega\ m_r\ s_p}{C_y}$	Mrstwn.	363	0.82	1.96

TABLE 6

Preliminary crosses involving chromosome III.

melanogaster ♀	*simulans* ♂	NUMBER OF FLIES	MEAN NUMBER OF BRISTLES MISSING	
			s_t	+
$\frac{rucuca}{C_{FL}\ C_{FR}}$	s_t	328	0.69	0.82
$\frac{s_t\ s_r\ e^4\ r_o\ c_a}{C_{IIILP}\ C_{IIIRP}\ l}$	s_t	418	0.8	0.50
$\frac{s_t\ s_r\ e^4\ r_o\ c_a}{C_{FL}\ C_{FR}}$	s_t	147	1.86	0.93
			$s_t\ p$	+
$\frac{thes}{C_{IIILP}\ C_{IIIRP}\ l}$	$s_t\ p$	200	0.49	0.25
$\frac{thes}{C_{FL}\ C_{FR}}$	$s_t\ p$	100	0.94	0.96
$\frac{res}{C_{FL}\ C_{FR}}$	$s_t\ p$	337	0.86	0.71
			s_s	+
$\frac{s_s\ h}{C_{FL}\ C_{FR}}$	s_s	910	0.23	0.33
$s_s\ h$	s_s	355	0.28	..

chromosome stock containing a dominant gene which could be used since any gene affecting the bristles had to be avoided. In this case stocks were used that contained a gene or genes corresponding to the *simulans*

genes. These *melanogaster* stocks were first balanced against a third chromosome crossover suppressor and then crossed to homozygous *simulans* males. The hybrids consisted of two classes and the effect of each chromosome could be observed.

The successful crosses using *melanogaster* stocks involving the X, second and third chromosomes are shown in tables 4, 5 and 6 with the mean number of bristles missing for each class. The columns headed by B, C_y, s_t, etc., represent the hybrids which showed Bar eye, Curly wing, scarlet eye, etc., but the actual *melanogaster* chromosome responsible for the effect is different in most cases as will be seen by examining the stocks used in each cross.

It is evident that the same *melanogaster* stock gave different averages with the different *simulans* males used in the crosses. This had not been expected, and further tests were made that will be described below.

CROSSES TO TEST CYTOPLASMIC DIFFERENCES IN *melanogaster* STOCKS

In order to test the effect of the cytoplasm on the bristles reciprocal matings were made between *melanogaster* stocks producing high and low numbers of missing bristles, and the F_1 females were then crossed to *simulans* males. Two stocks were obtained which could be used reciprocally since the recessive genes present would not affect the viability of the males. Females having the constitution $\frac{yU\ c_v\ v\ f}{s_c\ c_v\ v\ f}$ and $\frac{y\ U\ e_c\ c_t^6\ g^2}{s_c\ e_c\ c_t^6\ g^2}$ were crossed to males of several different *simulans* stocks, but none of the cultures produced flies. Many different numbers of males and females per culture were tried (STURTEVANT [1915] has shown there may be fighting between

TABLE 7

$$\frac{g_t bb''}{ClB}\ ♀ \times \frac{c_v}{dp^2 C_y}\ ♂ \rightarrow \frac{ClB}{+}\ \frac{dp^2 C_y}{+}\ ♀\ mel. \times Mrstwn.\ ♂\ sim.$$

CULTURE	NUMBER OF BRISTLES MISSING															
	C_y B				C_y +				+ B				+ +			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
112	86	3	2	0	77	7	3	0	62	7	1	0	55	4	1	0
121	51	10	5	0	57	5	1	0	32	5	2	1	33	4	3	0
122	30	6	1	0	27	5	3	0	22	7	1	0	33	7	1	2
Total	167	19	8	0	161	17	7	0	116	19	4	1	121	15	5	2
Mean values	0.18				0.17				0.21				0.22			

the males if two court the same female), and the wings and legs of the females were cut in the hope of making mating easier, but all the cultures failed (see STURTEVANT 1929). It is probable that mating did not take place, since many eggs were laid, but none hatched. Some of the females were dissected, but no sperm could be found in their receptacles This does not, however, prove conclusively that no mating took place, since the females were at least nine days old and the sperm may have been used up after a possible mating, but males were still alive in the bottles when the females were dissected and sperm should be found if a recent mating had occurred. These males were found to contain many sperm. Males of the constitution $y\ U\ e_e\ c_t{}^6\ g^2$ were crossed to *simulans* females, but again all the matings failed.

Of the second chromosome stocks $\frac{c_v}{d_p{}^2 C_v}$ males were crossed with $\frac{g_t\ bb''}{ClB}$ females and the $\frac{ClB}{+}\ \frac{d_p{}^2\ C_v}{+}$ F_1 females crossed with Morristown *simulans* males (table 7). The low values—much lower than those for either parent—are probably due to the recombination of modifiers through crossing the two stocks. The high average stock $\frac{b\ g_p\ a}{C_v\ R}$ was crossed reciprocally with the low average stock $s_e h$ and the $\frac{C_v}{+}\ \frac{s_e\ h}{+}$ F_1 flies used in crosses with sepia *simulans* males and females.

(A) $$\frac{b\ g_p\ a}{C_v R}\ ♀ \times \frac{s_e\ h}{s_e\ h}\ ♂ \rightarrow \frac{C_v}{+}\ \frac{s_e\ h}{+}\ ♀ \text{ and } ♂$$

(B) $$\frac{s_e\ h}{s_e\ h}\ ♀ \times \frac{b\ g_p\ a}{C_v\ R}\ ♂ \rightarrow \frac{C_v}{+}\ \frac{s_e\ h}{+}\ ♀ \text{ and } ♂$$

The F_1 females (A) and (B) have exactly the same chromosome constitution, but (A) females came from eggs with C_v cytoplasm and (B) females came from eggs with $s_e\ h$ cytoplasm. The males are alike except for the X and Y. The (A) males receive their X from the C_v stock and their Y from the $s_e\ h$ stock; the (B) males receive their X from the $s_e\ h$ stock and their Y from C_v. The different Y chromosomes can be seen to have no effect on the bristles of the hybrids, since the classes from both (A) and (B) crosses show practically the same values (table 8). This table also shows the hybrids from (A), and (B) females crossed with s_e *simulans* males differ from each other slightly or not at all. If the cytoplasm of the C_v stock was responsible for the high number of missing bristles we would expect the

TABLE 8

Mean number of bristles missing in crosses between s_e sim. and C_y s_e h mel.

melanogaster PARENT	*simulans* PARENT									
	s_e ♂ PRODUCING ♀ HYBRIDS					s_e ♀ PRODUCING MOSTLY ♂ HYBRIDS				
	NUMBER OF FLIES	C_y s_e	C_y +	+ s_e	++	C_y s_e	C_y +	+ s_e	++	NUMBER OF FLIES
$\frac{C_y}{+}\frac{s_e h}{+}$ from (A)	3372	0.25	0.24	0.18	0.20	♂ 1.24	1.62	0.39	0.63	5078
						♀ 0.56	0.55	0.15	0.28	379
$\frac{C_y}{+}\frac{s_e h}{+}$ from (B)	1731	0.23	0.18	0.16	0.17	1.29	2.01	0.40	0.66	2436
$\frac{C_y}{+}\frac{s_e h}{+}$ from (D)	1099	0.22	0.31	0.12	0.19	1.64	2.45	0.44	0.83	2088

hybrids from (A) to show a higher number missing than those from (B). The striking difference is that between the number of bristles missing in males compared with females and the separation of these values into four classes in the case of the males. The difference is seen to be due to the sex of the hybrid, since males and females which hatched from the same cultures (A ♂ × s_e *simulans* ♀) gave decidedly different values (table 8).

One may conclude that the differences between the two *melanogaster* stocks used are chromosomal in nature, rather than cytoplasmic.

THE USE OF *melanogaster* ATTACHED-X STOCKS

Since the hybrid males have more bristles missing than the females it was decided to study them in particular and to compare the hybrid males obtained by using *melanogaster* attached-X stocks with hybrid males from *simulans* mothers. A *melanogaster* stock was made up as follows:

$$\widehat{yy}\ ♀ \text{ from } \frac{p_n{}^2}{\widehat{yy}} \times \frac{B_l}{C_y\ s_p}\ ♂ \rightarrow \widehat{yy}\frac{C_y}{+}\ ♀$$

$$\widehat{yy}\frac{C_y}{+}\ ♀ \times \frac{res}{D_f\ 2C\ c_a}\ ♂ \rightarrow \widehat{yy}\frac{C_y}{+}\frac{D_f}{+}\ ♀$$

$$\text{(C)}\quad \widehat{yy}\frac{C_y}{+}\frac{D_f}{+}\ ♀ \times \frac{\text{II}}{\text{II}}\frac{s_e h}{s_e h}\ ♂ \rightarrow \widehat{yy}\frac{C_y}{\text{II}}\frac{s_e h}{D_f}\ ♀ \text{ and } \frac{C_y}{\text{II}}\frac{s_e h}{D_f}\ ♂.$$

The stock was kept by selecting the $\widehat{yy}$ C_y D_f females and crossing to s_e h males. Thus the stock contained the crossover suppressor including C_y over the normal second chromosome from s_e h and the third chromosome from s_e h over the crossover suppressor including D_f. Males and females are alike except for the X chromosome, but this does not enter into the constitution of the hybrids, since only male hybrids (with *simulans* X)

will be produced when either males or females from this stock are crossed to *simulans*. Many male hybrids were obtained by using $\widehat{yy}\ \frac{C_v}{\text{II}}\ \frac{s_e\ h}{D_f}$ females, but unfortunately the crosses (thirty-eight attempts) involving $\frac{C_v}{\text{II}}\ \frac{s_e\ h}{D_f}$ males failed.

TABLE 9

Mean number of bristles missing using $\widehat{yy}\ \frac{C_v}{II}\ \frac{s_e h}{D_f}$ ♀ *mel. (C).*

simulans MALE USED	NUMBER OF FLIES	$C_v +$	$C_v\ D_f$	$+ +$	$+\ D_f$
s_e	589	2.97	3.12	1.21	1.72
N. Orl.	661	2.82	2.91	1.25	1.60
Pas.	652	2.95	3.15	1.44	1.65
Mrstwn.	679	1.59	1.52	0.51	0.57

Table 9 shows the results of crosses between (C) females and males from four different *simulans* stocks. The hybrids from Morristown males are seen to give lower values than the others in all four classes. In this table the class labeled C_v+ corresponds to the $C_v\ s_e$ class of table 8. $C_v\ D_f$ corresponds to the C_v+ class, and so on, since the D_f chromosome now corresponds to the +third chromosome of table 8. Of course sepia appeared in the hybrids from the cross with s_e *simulans*.

Another *melanogaster* stock was made up as follows:

(D) $$\frac{B_l}{C_v\ s_p}\ ♀ \times \frac{\text{II}}{\text{II}}\ \frac{s_e\ h}{s_e\ h}\ ♂ \rightarrow \frac{C_v}{\text{II}}\ \frac{s_e\ h}{+}\ ♀ \text{ and } ♂.$$

These $\frac{C_v}{\text{II}}\ \frac{s_e\ h}{+}$ flies were like the $\widehat{yy}\ \frac{C_v}{\text{II}}\ \frac{s_e\ h}{D_f}$ flies with regard to the Y, to one third and to both second chromosomes. The male hybrids from $\frac{C_v}{\text{II}}\ \frac{s_e\ h}{+}$ *melanogaster* males would differ from the hybrids from $\widehat{yy}\ \frac{C_v}{\text{II}}\ \frac{s_e\ h}{D_f}$ females only in the chromosome containing D_f. They serve in a partial comparison of male hybrids derived from eggs containing different cytoplasm. Females from (D) were also compared with females from (A) and (B) in table 8. Little difference was expected in the comparison of the females, since they generally give low values with slight variations. The males from (C) and (D) (tables 9 and 8) differ in regard to missing bristles in each case except when Morristown male is crossed to (C) female. Considering the first three crosses it is seen that the difference in each class is consistent. Since the values for (D) males are with s_e *simulans* females,

the hybrids from (C) females by s_e males alone would be more nearly comparable. Here the differences suggest that something is active during development causing one group of hybrids to have more bristles missing than the other. This comparison is not exact, however, because the hybrids from (C) contain the D_f chromosome while the others contain the normal third chromosome. Considering the four classes of hybrids produced in these crosses and comparing the ratio of D_f to s_e (2.42 to 2.11) with that of + to s_e (in the cross with [D]: 1.65 to 0.99) it appears that any effect on the bristles by D_f is in the direction of reducing the number missing, thus reducing the differences in our comparison of crosses with (C) and (D) instead of increasing them.

A more direct comparison was made by using the following stocks:

(E) $\widehat{yy}$ ♀ from $\dfrac{p_n{}^2}{\widehat{yy}} \times \dfrac{b\ g_p\ a}{C_y\ R}$ ♂ → $\widehat{yy}\dfrac{C_y}{+}$ ♀ and $\dfrac{C_y}{+}$ ♂

(F) $\widehat{yy}$ ♀ from $\dfrac{t_a}{\widehat{yy}} \times \dfrac{b\ g_p\ a}{C_y\ R}$ ♂ → $\widehat{yy}\dfrac{C_y}{+}$ ♀ and $\dfrac{C_y}{+}$ ♂.

The males and females of (E) and of (F) have the same chromosome (autosome) constitution and when crossed with *simulans* females or males should produce only male hybrids. The hybrids will have different *melanogaster* Y, but this has been shown to have no effect on the bristles (see discussion page 161, and table 8). These hybrids should be exactly alike except

TABLE 10

Reciprocal crosses.

simulans STOCK	*melanogaster* STOCK					
	$\widehat{yy}\ C_y$ ♀ FROM (E)			C_y ♂ FROM (E)		
	NUMBER OF FLIES	MEAN NUMBER OF MISSING BRISTLES		NUMBER OF FLIES	MEAN NUMBER OF MISSING BRISTLES	
		C_y	+		C_y	+
yw♂ − ♀	58	2.70	1.50	135	2.51	0.47
s_e♂ − ♀	..	..	..	286	2.81	1.18
		$\widehat{yy}\ C_y$ ♀ from (F)			C_y ♂ from (F)	
yw♂ − ♀	146	3.50	1.65	207	2.10	0.37
s_e♂ − ♀	78	2.95	1.78	315	2.36	1.11
		$\widehat{yy}\ C_y$ ♀ from (G)			C_y ♂ from (G)	
b ♂ − ♀	314	5.04±0.17	3.52±0.19	1693	2.65±0.07	1.20±0.05
		$\widehat{yy}\ C_y$ ♀ from (H)			C_y ♂ from (H)	
b ♂ − ♀	583	4.32±0.15	3.10±0.12	1030	3.22±0.10	1.07±0.06

that one type is developed from *simulans* egg and the other from *melanogaster* egg. Any difference between the two types should be due to the effect of the egg cytoplasm on the development. The results of the crosses are shownin table 10. This table shows that in each case hybrids developing from *melanogaster* eggs had a higher number of bristles missing than hybrids of the corresponding class developing from *simulans* eggs. The chromosome constitution of the hybrids from the two crosses is identical.

Another test was made which shows a definite maternal effect on the bristles of the hybrids. An attached-X stock was made up in such a way that the male and female flies were known to have identical Y chromosomes. A double yellow female from $\frac{p_n{}^2}{\widehat{yy}}$ was mated to a $\frac{b\ g_p\ a}{C_y\ R}$ male and an F_1 *yy* C_y female and C_y male crossed. An F_2 male was then crossed back to an F_1 $\widehat{yy}$ C_y female. This stock will be designated as (G).

$$\widehat{XX}\underline{Y} \times XY\,\frac{b\ g_p\ a}{C_y\ R} \rightarrow \widehat{XX}Y\,\frac{C_y}{+} \times X\underline{Y}\,\frac{C_y}{+} \rightarrow \widehat{XX}\underline{Y}\,\frac{C_y}{+} + XY\,\frac{C_y}{+}$$

(G)
$$\widehat{XX}Y\,\frac{C_y}{+} \times XY\,\frac{C_y}{+} \rightarrow \widehat{XX}Y\,\frac{C_y}{+} + XY\,\frac{C_y}{+}$$

A similar stock was made up using a female from $\frac{t_a}{\widehat{yy}}$ and will be designated as (H).

Reciprocal crosses were made between each of the stocks (G) and (H), and black *simulans*. The hybrids were examined and a record kept of all the dorsocentral and scutellar bristles instead of recording no more than four as was done previously. These results are included in table 10. The differences between classes are in the same direction as those found with stocks (E) and (F).

EFFECT OF *melanogaster* CYTOPLASM

The results of all the above crosses in which the hybrids from reciprocal matings have their chromosome constitution identical or nearly so are arranged in table 11. The sex chromosomes are marked Xm for *melanogaster* X and Xs for *simulans* X. The Y comes from the *melanogaster* parent in each case. C_y indicates that the hybrids contained the *melanogaster* Curly chromosome, and + indicates the presence of the not-Curly chromosome. The other chromosomes are identical in each case and are not represented. The terms low, medium, high, etc., are purely arbitrary, based on the mean values shown.

The female hybrids developing from *simulans* eggs show a slightly higher mean value than those from the reciprocal cross. However, in other

TABLE 11

Results from reciprocal crosses.

HYBRIDS FROM *melanogaster* EGGS				HYBRIDS FROM *simulans* EGGS			
melanogaster ♀	CHROMOSOME CONSTITUTION	MEAN NUMBER OF MISSING BRISTLES		*melanogaster* ♂	CHROMOSOME CONSTITUTION	MEAN NUMBER OF MISSING BRISTLES	
$\frac{C_y}{+}\ \frac{s_e\ h}{++}$	Xm Xs C_y	Very low	0.24	$\frac{C_y}{+}\ \frac{s_e\ h}{++}$	Xm Xs C_y	Low	0.55
from (A)	Xm Xs +	Very low	0.19	from (A)	Xm Xs +	Very low	0.21
$\widehat{yy}\frac{C_y}{\text{II}}\ \frac{s_e\ h}{D_f}$	Ym Xs C_y	High	2.97	$\frac{C_y}{\text{II}}\ \frac{s_e\ h}{+}$	Ym Xs C_y	Medium	1.95
from (C)	Ym Xs +	Medium	1.46	from (D)	Ym Xs +	Low	0.52
$\widehat{yy}\,C_y$ from (E)	Ym Xs C_y	High	3.19	C_y from (E)	Ym Xs C_y	Medium	2.48
and (F)	Ym Xs +	Medium	1.65	and (F)	Ym Xs +	Low	0.89
$\widehat{yy}\,C_y$ from (G)	Ym Xs C_y	Very high	4.57	C_y from (G)	Ym Xs C_y	High	2.86
and (H)	Ym Xs +	High	3.25	and (H)	Ym Xs +	Medium	1.15

crosses using females from (A) values were obtained which approach 0.55, and in crosses with females from (D) values higher than this were obtained (see table 2). The male hybrids derived from (C) were not identical

TABLE 12

Influence of simulans male on bristle number.

simulans MALE	$\widehat{yy}$ ♀ FROM $\frac{p_n{}^2}{\widehat{yy}}$		$\widehat{yy}$ ♀ FROM $\frac{t_a}{\widehat{yy}}$	
	NUMBER OF FLIES	MEAN NUMBER MISSING BRISTLES	NUMBER OF FLIES	MEAN NUMBER MISSING BRISTLES
b	438	2.92	552	2.73
N. Orl.	870	2.26	770	1.93
s_e	528	2.28	1409	1.83
Pas.	948	1.81	634	1.89
St. Aug.	524	2.19	532	1.32
Mrstwn.	1424	0.78	1358	0.74
y w	..	..	118	2.53
y p_n	..	..	173	1.38

with those derived from (D) since one parent contained the D_f chromosome. As shown above the D_f chromosome influenced the bristles even less than the homologous normal chromosome. However, the best comparisons for the males are those involving the hybrids derived from (E), (F), (G) and (H). In (E) and (F) the two groups are identical in constitution

except for the origin of the *melanogaster* Y as mentioned above. The differences between the mean values 3.19 and 2.48, and the values 1.65 and 0.89 are certainly significant. In (G) and (H) the two groups of hybrids are identical in chromosome constitution since the stocks were made up in such a way that hybrids from both *melanogaster* males and females would contain the same chromosomes. Here the values for each class are higher, since all eight of the bristles were recorded. A comparison of these values is shown at the bottom of table 10.

TABLE 13

Comparison of black and Morristown stocks when mated to $\widehat{yy}$ ♀ *from* $\frac{t_a}{\widehat{yy}}$.

simulans MALE	NUMBER OF CULTURES	AVERAGE FLIES PER CULTURE	NUMBER OF BRISTLES MISSING					MEAN VALUE
			0	1	2	3	4	
b	4	138	72	56	80	83	261	2.73
Mrstwn.	10	136	791	282	164	76	45	0.74

In each case the hybrids from a *melanogaster* mother have more bristles missing than those from a *simulans* mother. The differences between these classes range from 6.1 to 15.6 times the mean error.

In every cross producing males, the hybrids which developed from *melanogaster* eggs showed a higher mean value than those which developed from *simulans* eggs. The females showed a difference in only one class and that in the opposite direction, but this disagreement has been explained above, partially at least.

INFLUENCE OF *simulans* STOCKS

It has been mentioned above that different results were obtained when various *simulans* stocks were crossed to the same *melanogaster* stock. (STURTEVANT [1929] did not find this difference, possibly because the *simulans* stocks he used produced a medium effect and also because he dealt mainly with female hybrids.) While most of the *simulans* stocks gave nearly equal numbers of missing bristles, one in particular (Morristown) gave a lower mean value. This difference is not easily detected in the female hybrids, but can be seen plainly in case of the males. Further crosses were made in order to compare male hybrids from various *simulans* stocks.

Males from four different *simulans* stocks were crossed with $\widehat{yy}\,\frac{C_v}{\text{II}}\,\frac{s_e\ h}{D_f}$ females from (C) and the male hybrids compared. These values are shown in table 9.

Males from eight different *simulans* stocks were crossed with $\widehat{yy}$ females from $\frac{p_n{}^2}{\widehat{yy}}$ and $\frac{l_a}{\widehat{yy}}$ *melanogaster* stocks. The results are shown in table 12. For comparison the actual numbers are shown for the cross of $\widehat{yy}$ females from $\frac{l_a}{\widehat{yy}}$ to black and Morristown stocks (table 13).

The hybrids from *simulans* females also differ in the number of bristles missing according to the *simulans* stock used. Females from six *simulans* stocks crossed to $\frac{C_y}{\text{II}}\frac{s_e\ h}{+}$ males from (D) produced male hybrids which differed from each other just as in the previous crosses. The results are given in table 14.

TABLE 14

Influence of simulans female on bristle number; $\frac{C_y}{II}\frac{s_e h}{+}\,\delta$ *from (D) used in each case.*

simulans FEMALE	NUMBER OF FLIES	MEAN NUMBER MISSING BRISTLES	
		C_y	+
St. Aug.	115	3.36	1.32
b	1270	2.70	0.79
s_e	2088	2.06	0.62
N. Orl.	844	1.84	0.29
Pas.	347	1.60	0.41
Mrstwn.	438	1.20	0.15

A comparison of the *simulans* stocks is shown in table 15. The *melanogaster* parent is listed at the top of each column and the various *simulans* stocks are arranged in the order of their effect on the bristles of the hybrids, the stock that gave most bristles missing being listed at the top of each column.

TABLE 15

Simulans stocks arranged in order of effect on the bristles of the hybrids.

EFFECT ON THE BRISTLES	$\frac{C_y}{\text{II}}\frac{s_e\ h}{+}\,♀$		$\frac{C_y}{\text{II}}\frac{s_e\ h}{+}\,\delta$		$\widehat{yy}\frac{C_y}{\text{II}}\frac{s_e\ h}{D_f}\,♀$		$\widehat{yy}$ ♀ FROM $\frac{p_n{}^2}{\widehat{yy}}$	$\widehat{yy}$ ♀ FROM $\frac{l_a}{\widehat{yy}}$
	C_y	+	C_y	+	C_y	+		
Greatest	*b*	*b*	St. Aug.	St. Aug.	Pas.	Pas.	*b*	*b*
	Mrstwn.	Mrstwn.	*b*	*b*	s_e	s_e	s_e	N. Orl.
	N. Orl.	N. Orl.	s_e	s_e	N. Orl.	N. Orl.	N. Orl.	Pas.
	s_e	s_e	N. Orl.	Pas.	Mrstwn.	Mrstwn.	St. Aug.	s_e
	Pas.	Pas.	Pas.	N. Orl.	..	..	Pas.	St. Aug.
Least	..	..	Mrstwn.	Mrstwn.	..	..	Mrstwn.	Mrstwn.

In each cross except one the values for the Morristown stock were the lowest. The discrepancy in this one case may be due to the small number of hybrids obtained. Black produced the greatest effect in all except one case where a small number of hybrids from St. Augustine stock showed a higher value. The other stocks were distributed at random between the highest and lowest values which suggests that they are practically equal in their effect on the hybrids. It is surprising that the black and Morristown stocks should differ so widely, since they are the most closely related of all the stocks used. The black mutation was found in the Morristown stock (STURTEVANT 1929) and the strain has never been out-crossed. Some change in one or both of the stocks must have taken place since then, or else the black mutation affects the bristles also. To test the second possibility, pair matings were made of black *simulans* flies, and the bristles counted. Of 391 offspring not one had less than four dorsocentral and four scutellar bristles, while some had extra scutellars. The mean number of bristles was 8.67 ± 0.04. It is probable that this excess of bristles is not directly related to the absence of them in the hybrids since here only the scutellars are duplicated while in the hybrids both dorsocentrals and scutellars are decreased in number.

The Morristown stock was then tested to see if a definite chromosome was the cause of the low bristle effect on the hybrids. It was compared with black to test the second chromosome and with sepia to test the third. A *melanogaster* stock ($\widehat{ff}$ *b* p_r *c* s_e *h*) was made up which had attached-X chromosomes bearing forked and was homozygous for the second chromosome characters black, purple, curved, and for the third chromosome characters sepia and hairy. Reciprocal matings were made between Morristown and *b* and Morristown and s_e, and the F_1 males in each case crossed to $\widehat{ff}$ *b* p_r *c* s_e *h* *melanogaster* females. This allowed a comparison of the normal second chromosome of Morristown with the *b* chromosome in one case, and of the normal third chromosome of Morristown with the s_e chromosome in the other. The effect of the Morristown X was tested in both cases. In these crosses all eight of the bristles were recorded. The results are shown in table 16.

The third chromosome of the Morristown stock does not differ from the s_e chromosome in its effect on the bristles of the hybrids since the values for these two classes are equal. The second chromosome of the Morristown stock is very little different from the *b* chromosome, since the values are nearly equal. In one case the difference between the black and normal classes is 0.77 ± 0.23 or 3.3 times the mean error, while in the other case it is only 0.56 ± 0.30 or 1.9 times the mean error. But both these differences

TABLE 16

Tests of Morristown stock.

TYPE OF CROSS	MEAN NUMBER MISSING BRISTLES	
	b	+
Mrstwn. ♀ × *b* ♂ → + ♂ *sim.* × $\widehat{ff}$ *b p_r c s_e h* ♀ *mel.*	2.59±0.18	1.82±0.14
b ♀ × Mrstwn. ♂ → + ♂ *sim.* × $\widehat{ff}$ *b p_r c s_e h* ♀ *mel.*	3.82±0.23	3.26±0.19
Difference	1.23±0.29	1.44±0.24
	s_e	+
Mrstwn. ♀ × s_e ♂ → + ♂ *sim.* × $\widehat{ff}$ *b p_r c s_e h* ♀ *mel.*	2.10±0.16	2.26±0.19
s_e ♀ × Mrstwn. ♂ → + ♂ *sim.* × $\widehat{ff}$ *b p_r c s_e h* ♀ *mel.*	3.56±0.18	3.48±0.19
Difference	1.46±0.24	1.22±0.27

are in the same direction, so it is possible that these two chromosomes differ slightly in their effect on the hybrids. The greatest difference between the Morristown and *b* and s_e stocks is due to the X chromosomes. The values from the reciprocal crosses differ in each class and show that the X chromosomes of the *b* and s_e stocks cause more bristles to be missing in the hybrids than the Morristown X chromosome. The differences are 4.2 and 6.0 times the mean error for the *b* stock and 6.1 and 4.5 times the mean error for the s_e stock.

BRISTLES OF THE PARENT SPECIES

Bristles were missing from some of the flies of every hybrid culture, regardless of the stocks which were used in the cross. Some *melanogaster* stocks gave higher numbers of missing bristles and some lower; also, different results were found to be due to the presence of different chromosomes. The chromosome containing Curly produces a greater effect than its homologue and the sepia hairy chromosome seems to produce less effect than its homologue. If specific genes acting on the bristles cause this effect in the hybrids, it would be suspected that the Curly chromosome contains more of these, or else certain ones producing greater effect than those in the sepia hairy chromosome. In that case the Curly chromosome might be expected to affect the bristles in *melanogaster* cultures. To test this $\frac{B_l}{C_y\, s_p}$ *melanogaster* females were mated to normal *melanogaster* males and 683 offspring examined. The mean number of bristles present for Curly flies was 8.01 ± 0.001 and for normal flies was 8.02 ± 0.008. Only one fly had less than

eight bristles and in that case it looked as though the bristle had been present but was broken off. Homozygous stock of sepia hairy would be expected to have some of the bristles absent, since it would be homozygous also for the genes causing the missing bristles in the hybrids. The bristles of 304 sepia hairy flies (from pair matings) were examined and the mean number of bristles present was found to be 8.64 ± 0.04. Not one fly had less than eight bristles and none had extra dorsocentrals. The extra bristles were scutellars and in most cases anterior scutellars. Duplication of these bristles was found to be one manifestation of the gene for hairy (MOHR 1922). The black *simulans* stock was tested in the same way, and the mean number of bristles present was found to be 8.67 ± 0.04. Hybrids from black *simulans* crossed to *melanogaster* flies showed the greatest number of missing bristles.

If specific genes cause missing bristles in the hybrids they do not produce a noticeable effect on *melanogaster* flies either when in heterozygous or in homozygous condition. The black *simulans* flies that were examined had extra bristles, while hybrids from these had many bristles missing.

DISCUSSION OF RESULTS

The effect on the bristles is not strongly correlated with other developmental abnormalities that appear in the hybrids. Many females appear to be abnormal in one way or another, but few bristles are missing. Males usually have a normal appearance, but have many bristles missing. The hybrid females often have rough eyes (especially those from certain *simulans* stocks) while the eyes of the hybrid males are normal or practically so. Hybrid females hatching from the same culture with males have rough eyes and the eyes of the males appear perfectly normal. At high temperature (29°C) female hybrids rarely develop past the pupal stage, while males emerge and appear normal. Males emerging at 29°C have fewer bristles missing than those emerging at 22°C. The abdomen of the females is more often abnormal than the abdomen of the males, and flies with abnormal abdomens may have all the bristles present.

The missing bristles are evidence of imperfect coördination of the genes of the two species. Genetic differences between the species were shown also in the appearance in the hybrids of the dominant *melanogaster* characters Bar and Lobe (MORGAN 1929) and Delta (STURTEVANT 1929). In respect to facet number Bar differs only slightly from normal eye in the hybrids and Lobe cannot be distinguished at all. The larger eye of *simulans* is probably responsible for part of this difference. The Delta character due to the *melanogaster* gene is less extreme in the hybrids than in pure *me-*

lanogaster, and that due to the *simulans* Delta gene is more extreme in the hybrids than in pure *simulans*.

Differences between hybrids from reciprocal species crosses have been attributed to the effect of the cytoplasm of the egg. In the present work hybrids developing from eggs containing *melanogaster* cytoplasm have more bristles missing than hybrids developing from eggs with *simulans* cytoplasm, even though the chromosomes in each case are identical. This may be interpreted as meaning that the chromosomes in the *melanogaster* egg have produced some effect on the cytoplasm before fertilization. Development might then be slightly different from that of a similar gene complex developing in a *simulans* egg. Whether the cytoplasm itself (independently of the chromosomes) causes any difference in development cannot be tested, since the hybrids have been found to be completely sterile (STURTEVANT 1920). Purely cytoplasmic inheritance is not probable, however, since the results show that the differences among the *melanogaster* stocks can all be accounted for in terms of chromosomes.

A "prematuration" (maternal) effect was considered by MORGAN (1912, 1915) as a cause of irregularities in the inheritance of rudimentary wing in *Drosophila melanogaster*. Miss LYNCH (1919) found this to be a cause of the partial sterility of homozygous rudimentary or fused females. Miss REDFIELD (1924, 1926) reported a sex-linked lethal effect which acted only when the mother was homozygous for a second chromosome gene. The females died chiefly in the egg stage due to the influence of the maternal genetic composition on the eggs before they left the mother's body. GABRITSCHEVSKY and BRIDGES (1928) found an enhancer of giant which showed a maternal effect on the eggs of females homozygous for the enhancer. STURTEVANT (1923) explained the inheritance of coiling in Limnaea as being due to the genetic complex of the unreduced egg regardless of the constitution of the offspring. DIVER, BOYCOTT and GARSTANG (1925) and BOYCOTT, DIVER, GARSTANG and TURNER (1930) have shown this to be the case since the coiling is delayed a generation in inheritance.

SUMMARY

1. In crosses between *Drosophila melanogaster* and *D. simulans* about 50 percent of the hybrids have bristles missing that are present in both parents. Bristles are missing from some of the hybrids of every cross.

2. The *melanogaster* stocks differ in their effect on the number of missing bristles in the hybrids. These differences are chromosomal rather than cytoplasmic in inheritance.

3. The *simulans* stocks differ in their effect on the hybrids. Black pro-

duced the greatest effect and Morristown the least. The other stocks that were tested gave generally the same values for missing bristles and are considered to be practically equal in their effects on the hybrids.

4. The difference between the black and Morristown stocks was found to be due chiefly to the X chromosomes of the two stocks.

5. The effects of the various *simulans* stocks on the bristles of the hybrids appear in hybrids from *simulans* mothers and in those from *simulans* fathers.

6. Male hybrids show the bristle effect more than do female hybrids. This difference cannot be due to external conditions since males and females hatching from the same culture have different numbers of missing bristles.

7. Male hybrids from attached-X *melanogaster* females were compared with male hybrids from *simulans* females. Those developing from *melanogaster* eggs had more bristles missing than hybrids developing from *simulans* eggs, although the chromosome constitution of the hybrids was identical. This maternal effect may be due to the influence of the chromosomes on the cytoplasm of the egg before the egg leaves the mother's body.

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STUDIES ON SELF-STERILITY. IX. THE BEHAVIOR OF CROSSES BETWEEN SELF-STERILE AND SELF-FERTILE PLANTS

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	175
Experimental work	179
The F_1 generation of the combination *N. Langsdorffii*×*N. Sanderæ*	179
F_1 (*N. Langsdorffii*×*N. Sanderæ*)×*N. Sanderæ*	180
N. Sanderæ×F_1 (*N. Langsdorffii*×*N. Sanderæ*)	182
The F_2 generation of the cross *N. Langsdorffii*×*N. Sanderæ*	184
Discussion and interpretation of the results	184
The cytoplasmic factor	191
The combination *N. Sanderæ*×*N. Langsdorffii*	192
The inheritance of certain color factors	193
Corolla color due to the interaction of *C* and *P*	193
The inheritance of the *P* factor	195
Inheritance of anther color and pollen color	196
Inheritance of green vs. non-green corolla	198
SUMMARY	199
ACKNOWLEDGMENT	201
LITERATURE CITED	201

INTRODUCTION

Nearly twenty years ago COMPTON (1912, 1913) studied crosses between self-fertile and self-sterile plants of *Reseda odorata* which had previously bred true to type. Self-fertility was dominant; and the members of the F_1 generation, when selfed, threw self-fertile plants and self-sterile plants in a ratio approximately 3 to 1.

Some time later (EAST 1919), I reported crosses between the self-fertile species *Nicotiana Langsdorffii* and the self-sterile species *N. Sanderæ* (then called *N. Forgetiana*) which appeared to corroborate COMPTON'S work. In reciprocal crosses, self-fertility was dominant. Selfed F_1 plants produced 144 self-fertile and 37 self-sterile plants, a ratio of 3.8 to 1. A cross between *N. Langsdorffii* and *N. alata* yielded similar results. The self-sterile segregates of both crosses bred true to self-sterility; but in the first cross there was an indication of the presence of a subsidiary pair of allelomorphs, *Dd*, of such a nature that the presence of *D* caused a considerable amount of pseudo-fertility (small capsules with a few good seeds).

TERAO (1923) obtained comparable results with *Petunia violacea*. BAUR (1919) also obtained similar results in a cross between the self-fertile *Antirrhinum majus* and the self-sterile *A. molle*. The F_2 population consisted of 190 self-fertile and 22 self-sterile plants.

These investigations indicated that the behavior of self-fertile plants when crossed with self-sterile plants is so simple as to be unworthy of further consideration. When proof was obtained (EAST and MANGELSDORF 1925) that the behavior of self-sterile plants of *N. Sanderæ* and *N. alata* in intra-specific crosses may be interpreted by the so-called oppositional hypothesis, however, I began to suspect that the apparent simplicity was illusory. When the oppositional hypothesis was found to hold for *Veronica syriaca* (FILZER 1926, LEHMANN 1926), for *Antirrhinum Segovii* (FILZER 1926, EAST 1926, SIRKS 1926), for *Verbascum phœniceum* (SIRKS 1926), and for *Capsella grandiflora* (SHULL 1926), these suspicions were crystallized. If, in a cross between plants carrying the self-sterility allelomorphs S_1S_3 and S_2S_3, equal quantities of plants S_1S_2 and S_3S_2 are obtained because only male gametes bearing the factor S_2 will grow rapidly enough to function, then it seemed probable that in selfed plants of the constitution S_fS_1—such as would probably be formed when *N. Langsdorffii* (S_fS_f) is crossed with *N. Sanderæ* (S_1S_1)—only pollen tubes bearing the factor S_f would function. In this case the F_2 generation would consist of equal quantities of plants S_fS_f and S_fS_1. No self-sterile plants would be obtained.

To test these suspicions, a new series of crosses was made between *N. Langsdorffii* and *Sanderæ*. The results are reported in this paper; and, as will appear, the more recent assumption was verified. Under the simplest set-up of the problem, selfed F_1 plants having such a constitution as S_fS_1 can not give self-sterile plants in the F_2 generation. The S_1 pollen tubes do not grow rapidly enough to fertilize.

How, then, can the earlier results of COMPTON, of BAUR, of TERAO, and of EAST be interpreted? The simplest way out of the difficulty is to assume that each investigator recorded as self-pollinations a series of pollinations that were actually intercrosses between F_1 plants. Two F_1 plants S_fS_1 and S_fS_2, if intercrossed, would give a population consisting of 3 self-fertile plants to 1 self-sterile plant. One is loath to accept such an explanation, however, for three reasons. First, it is unlikely that four reasonably careful workers made similar blunders of record. Second, my own data cards show five separate entries as *selfings* where they should read *intercrossings* under this explanation. One such error might be possible; I can not think that five are possible. Third, with this explanation, the self-sterile plants of each F_2 population would all belong to one intra-sterile class. In the illus-

tration given above, each plant would have the constitution S_1S_2. This is not in accord with the facts. In these earlier records there are several cases where the F_2 plants were apparently compatible with each other and therefore should be expected to have different genetic constitutions. For these reasons, another explanation of the aberrant early records must be sought.

In certain cases of complicated behavior, such as those recorded by EAST and PARK (1917), it is probable that plants carrying moderately rapid-growing *S* factors were involved. Such plants, frequently giving some seed in incompatible matings, were known to have been present in our cultures of that date. They were later discarded in favor of plants carrying slow-growing *S* factors, since the behavior of such plants could be analyzed more easily. It is unlikely, however, that the pseudo-fertility accompanying incompatible matings in instances where moderately rapid-growing pollen tubes are concerned is the entire basis for the results reported in 1917 when only one pair of oppositional factors of the ordinary type is present. It is more probable that other variables are involved.

We know of two such variables which have the type of action required. For example, ANDERSON and DE WINTON (1931) have described a gene at the *S* locus which causes behavior quite different from that usually encountered. This factor was discovered in a plant of *N. alata*. When plants carrying this gene were crossed with *N. Langsdorffii*, the F_1 generation consisted of self-fertile and self-sterile plants in equal numbers; and these plants behaved in an extraordinary manner. The self-sterile members of the population were compatible reciprocally with *N. alata*, but were compatible only as males with *N. Langsdorffii*. ANDERSON and DE WINTON interpreted this behavior by postulating a factor S_F, carried by the exceptional *alata* plant, which not only has the characteristics of the ordinary factors located at the *S* locus, but also has the property of inhibiting the growth of pollen tubes carrying the full fertility factor S_f. Four types of test matings were made, confirming the hypothesis.

Such a factor would not ordinarily provide for the production of self-fertile and self-sterile segregates in the F_2 generation when any given F_1 plant of a cross between self-fertile and self-sterile biotypes is self-pollinated. The self-fertile F_1 plants should give S_fS_f and S_fS_n plants—all self-fertiles—in equal numbers. The self-sterile F_1 plants, S_fS_F, could not ordinarily be self-pollinated. But if, under optimum conditions, the pollen tubes concerned grow somewhat more rapidly than usual, then the F_2 generation should consist of $S_fS_f+2S_fS_F+S_FS_F$ plants; and these plants might have reactions which would make the family appear to consist of self-sterile and self-fertile plants in varying proportions.

The case is cited because it shows that we do not have a complete knowledge of the behavior of all of the allelomorphs of the *S* locus. It is by no means improbable that a plant carrying some unknown *S* factor was crossed with *N. Langsdorffii* in our earlier crosses and that the behavior of the plants obtained was such as to give both self-fertiles and self-steriles in the F_2 generation.

A second point to be kept in mind is that modifying factors exist which shift the action of the factors at the *S* locus. I found evidence of one such factor in *N. Langsdorffii* (EAST 1919). BRIEGER (1927) isolated another such factor in *N. Sanderæ*, which he called the "Pseudofertility factor P." ANDERSON and DE WINTON (1931) also found indications of such factors. And several are discussed in the present paper. The most interesting case of this kind, however, was reported by KAKIZAKI (1930) in experiments on the common cabbage, *Brassica oleracea capitata*. KAKIZAKI found that cabbage varieties contained a series of oppositional allelomorphs S_1, S_2, and S_3, which behaved like those found in *N. Sanderæ*. In addition, he found evidence of the presence of stimulating allelomorphs T_1 and T_2. The mutual influence of the *T* factors in pollen tubes and style is just the opposite of that of the *S* factors. A T_1T_1 style, for example, accelerates the growth of T_1 pollen tubes, though it does not affect the growth of the T_2 pollen tubes. Further, though two *S* factors are more active than one *T* factor, two similar *T* factors are more active than one *S* factor. This situation gives rise to very complicated results, which are presented and analyzed in Doctor KAKIZAKI'S paper. Thus, in KAKIZAKI'S experience, plants such as $S_1S_2T_1T_1$ will produce, on selfing, populations which are half self-fertile and half self-sterile. Plants having the constitutions $S_1S_1T_1T_1$ and $S_2S_2T_1T_1$ will be self-sterile; but if the *T* factor is more effective on one *S* factor than on the other, the F_2 ratios may be distorted.

It is obvious that with simple subsidiary factors showing no dominance or epistasis, with simple subsidiary factors showing dominance or epistasis, with subsidiary factors where two doses dominate the action of the primary factor while one dose does not, and with factors which act not only against the identical factor when present in both style and pollen tube but also against a second factor (S_1 against both S_1 and S_2), as has been reported by SIRKS (1926), it is a simple matter to find conditions in which a self-fertile plant will throw self-fertile and self-sterile segregates. Perhaps the simplest conditions are those in which the constitution of the F_1 plant is $S_fS_1T_aT_r$, where S_f is the ordinary fertility factor, S_1 is a self-sterility factor, T_a is an accelerating factor, and T_r is a retarding factor. If gametes of the constitution S_nT_r do not function, there will be two self-steriles in

every twelve plants. They will have the constitutions $S_1S_1T_aT_a$ and $S_nS_nT_aT_r$. These two types might be expected to cross together.

Finally I should like to call attention to a very pertinent suggestion made by CRANE and LAWRENCE (1931). These authors have found that self-sterile diploid plums and cherries behave essentially the same as the self-sterile plants of *N. Sanderæ*. In tetraploid varieties, however, incompatible types are less frequently met; variable results are common; and one-way compatibility is common. In a tetraploid cross, as for example $S_1S_1S_2S_2 \times S_1S_1S_2S_3$, remark the authors, it is conceivable that the plant carrying the S_3 factor might be compatible as male but not as female.

It is unnecessary to assume tetraploidy to explain the results obtained earlier (EAST 1919) in crosses between *N. Langsdorffii* and *N. Sanderæ*. It may be that tetraploids are found in these species; but in numerous chromosome determinations only 9 pairs have been discovered. In crosses, moreover, these 9 pairs behave in a perfectly normal manner, as if one were dealing with varietal rather than specific differences. As to whether the other aberrant results which we have been considering have been brought about by polyploidy it is difficult to say. Tetraploidy is common in *Petunia violacea* (DERMEN 1931) and may have been present in TERAO'S stock. It is not rare in Brassica, though it has not been found in any of the varieties of *Brassica oleracea* (GAISER 1930) such as were used by KAKIZAKI. The same may be said for *Antirrhinum majus* and for *A. Segovii*, both of which have always been found to have 8 pairs of chromosomes. Since the chromosome numbers of Verbascum species are 16, 24, and 32, however, one may assume that the 16-chromosome species are tetraploids. It is possible, therefore, that the situation in *V. phœniceum* is complicated on this account, as CRANE and LAWRENCE suggest.

EXPERIMENTAL WORK

The F_1 generation of the combination "N. Langsdorffii × N. Sanderæ"

Several crosses were made between *N. Langsdorffii* and *N. Sanderæ*. The cross was slightly easier to make when *N. Langsdorffii* was used as female. Extensive tests were made on four combinations. *Langsdorffii* 1 was crossed with one *Sanderæ* plant homozygous for S_1 and with a second *Sanderæ* plant homozygous for S_3. *Langsdorffii* 3 was crossed with the same two *Sanderæ* plants. The *Sanderæ* plant having the constitution S_3S_3 was in some ways a poor choice, for this factor, when in a homozygous condition, has an adverse effect on the development of the plants. The leaves

are curled and twisted; the stems are short, stout, and somewhat gnarled. The plants are moderately vigorous, though subnormal, after they reach the flowering stage, but are very delicate as seedlings. With careful treatment, however, the loss of seedlings is small. The type was selected because homozygous segregates can be detected without making the usual pollination tests.

The F_1 plants were self-fertile, were fertile reciprocally in backcrosses with *Langsdorffii*, and were completely fertile with the pollen of all plants homozygous for allelomorphs at the *S* locus except the plants homozygous for the factor that had entered into the original combination. Thus F_1 plants of *Langsdorffii* $1 \times$ *Sanderæ* S_1S_1 were sterile to the pollen of S_1S_1 plants, and F_1 plants of *Langsdorffii* $3 \times$ *Sanderæ* S_3S_3 were sterile to the pollen of S_3S_3 plants. One may conclude, therefore, that S_n pollen does not ordinarily grow rapidly enough to function in S_fS_n styles. S_f is the full fertility factor carried by *N. Langsdorffii*; S_n denotes any of the other sterility allelomorphs of the *S* locus.

$$F_1 \text{ (}N.\ Langsdorffii \times N.\ Sanderæ\text{)} \times N.\ Sanderæ$$

Fourteen backcrosses were made using the F_1 plants as females and various *Sanderæ* plants as males. The plants of the resulting populations were tested with the pollen of selected test plants. These results are set forth in table 1. The first column gives the laboratory number of the family. The second column gives the parentage. For example, family 103 was produced by crossing an F_1 plant 1EG—which was a cross between *Langsdorffii* 1 (S_fS_f) and *Sanderæ* 29FA (S_1S_1)—with pollen from *Sanderæ* 30EA (S_2S_2). The third column gives the results obtained when the plants were selfed. The remaining columns record the results obtained when the plants were tested with various *S* testers. Two families, 112 and 117, backcrosses of the type $S_fS_1 \times S_1S_1$, were incompatible matings, and were obtained by pollinating buds about two days before the flowers were due to open. The other families were the result of compatible matings made in the ordinary way.

The most significant fact in this table is the production of male-sterile plants. These plants ordinarily produced no pollen whatever, although a few plants which appeared to belong to this group—about a dozen altogether—produced about five percent of functional pollen. These plants are evidently those which contain two *S* factors and correspond to the self-sterile plants of the reciprocal mating. They become male sterile through the action of a cytoplasmic factor brought in by *N. Langsdorffii* when used as the female in crosses. The ratio of self-fertiles to male steriles, omitting the two families produced by bud pollination and family 120, is

266 to 232. Presumably the male-sterile class is absent from family 120 because the zygotes S_3S_1 in combination with *Langsdorffii* cytoplasm are not viable. BATESON and GAIRDNER (1921) found a similar case of male-

TABLE 1

F_1 (N. Langsdorffii×N. Sanderæ)×N. Sanderæ.

FAMILY NUMBER	PARENTAGE	SELFED		×S_1S_1		×S_1S_2		×S_1S_1 ×S_1S_2				×S_2S_2	
		RESULTS	NUMBER	+	−	+	−	+ +	+ −	− +	− −	+	−
103	1EG(S_fS_1)×30EA(S_2S_2)	+	27	27	0	25	0	25	0	0	0	11	8
		♂ sterile	32	0	32	23	7	0	0	22	7	9	8
105	2EG(S_fS_1)×45L$_1$(S_2S_2)	+	23	23	0	20	0	21	0	0	0	1	15
		♂ sterile	26	0	26	5	21	0	0	5	21	1	23
108	1EK(S_fS_1)×45L$_1$(S_2S_2)	+	32	32	0	31	0	31	0	0	0	2	10
		♂ sterile	23	0	23	4	19	0	0	4	19	0	21
112	2EK(S_fS_1)×29FA(S_1S_1)*	+	30	0	0	..	..	..	..	..	..	..	..
		♂ sterile	15	0	2	..	..	..	..	..	..	..	..
114	2EK(S_fS_1)×30EA(S_2S_2)	+	33	33	0	33	0	33	0	0	0	..	..
		♂ sterile	27	0	27	15	12	0	0	15	12	9	2
				×S_3S_3		×S_1S_3		×S_3S_3 ×S_1S_3				×S_2S_2	
117	1EO(S_fS_3)×1DD(S_3S_3)*	+	29	0	28	..	..	..	..	..	..	..	..
		♂ sterile	14	0	14	..	..	..	..	..	..	..	..
120	2EO(S_fS_3)×29FA(S_1S_1)	+	30	30	0	19	0	19	0	0	0	..	..
		♂ sterile	0	0	0	0	0	0	0	0	0	..	..
				×S_3S_3		×S_2S_3		×S_3S_3 ×S_2S_3				×S_2S_2	
121a	2EO(S_fS_3)×26EA(S_2S_2)	+	12	12	0	9	0	9	0	0	0	..	..
		♂ sterile	11	7	3	5	4	3	3	1	1	..	..
121b	2EO(S_fS_3)×30EA(S_2S_2)	+	6	6	0	6	0	6	0	0	0	..	..
		♂ sterile	5	2	3	2	1	1	1	1	0	..	..
123	3EO(S_fS_3)×30EA(S_2S_2)	+	30	..	..	30	0	..	..	..	..	5	4
		♂ sterile	18	..	..	16	2	..	..	..	..	8	5
125	6EO(S_fS_3)×45L$_1$(S_2S_2)	+	12	5	1	11	0	4	0	1	0	..	..
		♂ sterile	18	1	13	6	12	1	0	4	9	..	..
127	7EO(S_fS_3)×45L$_1$(S_2S_2)	+	33	11	0	33	0	11	0	0	0	1	4
		♂ sterile	29	0	3	12	16	0	0	0	2	3	20
131	1EP(S_fS_3)×45L$_1$(S_2S_2)	+	21	..	..	20	1	..	..	..	..	0	7
		♂ sterile	21	..	..	12	8	..	..	..	..	1	15
133	2EP(S_fS_3)×30EA(S_2S_2)	+	32	..	..	28	0	..	..	..	..	10	3
		♂ sterile	30	..	..	23	1	..	..	..	..	20	5

* Bud pollination

sterile production in crosses between procumbent and tall varieties of flax (*Linum usitatissimum*). CHITTENDEN and PELLEW (1927) and CHITTENDEN (1927) have given an analysis of the facts which shows that the gene *T* from tall flax when homozygous in cytoplasm from procumbent flax gives rise to male steriles. LEHMANN and SCHWEMMLE (1927) and LEHMANN (1928) have reported a similar occurrence in Epilobium.

Before examining the results obtained in the test pollinations, it may be well to call attention to the fact that these tests have inherent deficiencies which can be eliminated only by a large number of repetitions. Approximately one percent of matings recorded as sterile are in error. They are matings which would have shown fertility had they been made several times but which appeared to show sterility because insufficient pollen was used or because the pollen did not adhere. From one-tenth of one percent to three percent of the matings recorded as fertile are errors of record owing to "pseudofertility." That is to say, a few pollen tubes sometimes function in incompatible matings. The error is not over one-tenth of one percent when S_1S_1 pollen is used, is perhaps one percent when S_3S_3 pollen is used, and is probably one-half of one percent when S_2S_2 pollen is used, on pure *Sanderæ* plants. Many of the matings reported here, where more variables are involved than with pure *Sanderæ* plants, were made several times, and nearly forty percent of them were made twice; but since it was necessary to classify the remainder on the basis of a single pollination, it is clear that all of the matings made can not be expected to check with each other.

Even though these facts are given due weight, however, it is obvious that the reactions are not so precise as those obtained when dealing with the behavior of self-sterile plants only (see EAST and YARNELL 1929). S_1 pollen behaves as expected. S_2 pollen, on the other hand, functions much more frequently than it should in incompatible matings—matings with male-sterile plants. The same remark applies to S_3 pollen, though the frequency with which S_3 pollen functions in incompatible matings is less than half the frequency with which S_2 pollen functions. Moreover, there is a consistency in the aberrant behavior of the S_2 and the S_3 pollen in families of similar derivation which leads to the idea that *N. Langsdorffii* has brought in factors which modify their behavior. The aberrant behavior is not due to the properties of the test plants themselves, for they had been shown to behave normally in numerous tests where only *Sanderæ* plants were involved.

N. Sanderæ$\times F_1$(*N. Langsdorffii*$\times$*N. Sanderæ*)

Twelve backcrosses were made using various *Sanderæ* plants as females and plants from the four F_1 populations as males. The results are brought

together in table 2. These crosses are, in the main, reciprocals of those reported in table 1. Family 113, involving only S_1, was obtained by bud-pollination; the other families were produced by normal cross-pollinations.

TABLE 2

N. Sanderæ×F_1 (*N. Langsdorffii*×*N. Sanderæ*).

FAMILY NUMBER	PARENTAGE	TYPE	SELFED		×S_1S_1		×S_2S_2		×S_1S_1 ×S_2S_2				×S_3S_3	
			+	−	+	−	+	−	+ +	+ −	− +	− −	+	−
104	45L$_1$(S_2S_3)×1EG(S_1S_1)	S_2S_1	41	0	32	0	41	0	32	0	0	0	6	24
		S_2S_1	7	13	0	18	10	10	0	0	8	10	3	15
106	45L$_1$(S_2S_3)×2EG(S_1S_1)	S_2S_1	29	0	26	1	28	0	25	0	1	0	..	..
		S_2S_1	3	33	0	36	5	31	0	0	5	31	..	..
109	45L$_1$(S_2S_3)×1EK(S_1S_1)	S_2S_1	27	0	14	0	25	0	14	0	0	0	3	10
		S_2S_1	6	13	0	16	3	16	0	0	2	14	4	12
113	29FA(S_1S_1)×2EK(S_1S_1)	S_1S_1	9	0	1	0	..	..	..	..	..	..	..	..
		S_1S_1	0	9	0	7	..	..	..	..	..	..	..	..
115a	26EA(S_2S_3)×2EK(S_1S_1)	S_2S_1	15	0	15	0	9	0	4	1	0	0	..	..
		S_2S_1	4	5	1	8	3	6	0	0	3	6	..	..
115b	30EA(S_2S_3)×2EK(S_1S_1)	S_2S_1	4	0	4	0	4	0	4	0	0	0	..	..
		S_2S_1	0	9	0	6	0	9	0	0	0	6	..	..
					×S_2S_3		×S_2S_3		×S_2S_3 ×S_2S_3				×S_2S_3	
122	26EA(S_2S_3)×2EO(S_1S_3)	S_2S_1	41	0	37	4	..	..	..	..	..	..	..	..
		S_2S_3	0	6	3	3	0	3	0	0	0	3	..	..
124	30EA(S_2S_3)×3EO(S_1S_3)	S_2S_1	27	0	22	0	..	..	..	..	..	..	..	..
		S_2S_3	10	11	2	19	..	..	..	..	..	..	0	8
126	45L$_1$(S_2S_3)×6EO(S_1S_3)	S_2S_1	44	0	..	..	37	2	..	..	..	..	12	17
		S_2S_3	0	16	..	..	3	11	..	..	..	..	3	10
128	45L$_1$(S_2S_3)×7EO(S_1S_3)	S_2S_1	45	0	..	..	37	0	..	..	..	..	10	23
		S_2S_3	2	15	..	..	1	15	..	..	..	..	0	15
132	45L$_1$(S_2S_3)×1EP(S_1S_3)	S_2S_1	47	0	..	..	47	0	..	..	..	..	14	23
		S_2S_3	4	10	..	..	1	13	..	..	..	..	1	10
134	30EA(S_2S_3)×2EP(S_1S_3)	S_2S_1	32	0	22	0	32	0	12	0	0	0	..	..
		S_2S_3	12	12	0	21	14	10	0	0	12	8	9	3

In these crosses one should find populations consisting of one-half self-fertiles and one-half self-steriles. This was not the case, however; there was a marked deficiency of self-steriles, especially in the populations where S_3 was brought in by the pollen. A considerable proportion of this deficiency is due to the fact that some of the S_2S_1 plants and some of the S_2S_3 plants, which should be self-sterile, are self-fertile, probably because some S_2 pollen functions. Many of these plants containing two S genes can be identi-

fied by the tests. The tests involving S_1 are the most reliable because S_1 shows no pseudofertility. In fact, it is with extreme difficulty that seeds are obtained after bud-pollination in incompatible matings where S_1 pollen is used. When compatibility or incompatibility with S_1 pollen is the chief criterion used for separating S_2S_f plants from S_2S_1 plants, the proportion is 116 to 93. Though 20 of these 93 S_2S_1 plants were self-fertile, they were all sterile to S_1S_1 pollen, with one exception. This one plant was classified as S_2S_1 because it was sterile to S_1S_2 pollen. Further tests would probably have shown that the plus reaction to S_1S_1 was an error. A goodly number of these plants were fertile to S_1S_2 pollen and to S_2S_2 pollen, thus showing that S_2 often reached the micropyle and fertilized the egg, though such reactions were not to be expected.

In the populations where S_2 and S_3 were involved, classification into the two types S_2S_f and S_2S_3 was made on all the data available, but chiefly by the reaction to S_3S_3 pollen. Here again S_2 pollen has a noticeable tendency to function in incompatible matings. S_3 pollen exhibits the same tendency, though in a smaller degree.

The F_2 generation of the cross "N. Langsdorffii×N. Sanderæ"

Ten straight F_2 families of the cross *N. Langsdorffii*×*N. Sanderæ* were produced by selfing F_1 plants. Six families came from normal self-pollinations; four families came from bud-pollinations. In the populations resulting from normal self-pollinations, one should expect the two classes S_fS_f and S_fS_1 (or S_fS_3) in equal numbers. All of the plants should be self-fertile, and this proved to be the case. Half of the plants, however, should be sterile to S_1S_1 (or S_3S_3) pollen, and this did not prove to be true. In the populations resulting from bud-pollinations, one should expect the classes $S_fS_f+2S_fS_1$ (or S_fS_3)$+S_1S_1$(or S_3S_3). Three-fourths of the plants, therefore, should be sterile to S_1S_1(or S_3S_3) pollen, and one-fourth should be male sterile. Neither of these expectancies was realized.

Discussion and interpretation of the results

Since the various test plants containing genes S_2 and S_3 which were used on the thirty-six families produced from eleven F_1 plants of the cross *N. Langsdorffii*×*N. Sanderæ* showed reactions which were quite different from those exhibited on straight *Sanderæ* plants, one must assume that genes are carried by *N. Langsdorffii* which act as modifiers. *N. Langsdorffii* also carries a full fertility factor at the *S* locus—namely, S_f—and a cytoplasmic factor which causes a zygote bearing two *S* factors other than S_f

(in so far as tests were made) to become male sterile. The justice of these assumptions will be manifest to the reader if he will refer to the clear-cut, precise results reported in *Studies on self-sterility VIII* (EAST and YARNELL 1929). The tests reported here do not have the regularity and consistency which was to have been expected on the basis of effective fertility-sterility factors located only at the *S* locus. These tests are understandable, how-

TABLE 3

The F_2 generation of N. Langsdorffii×N. Sanderæ.

FAMILY NUMBER	FAMILY	TESTED WITH	RESULT AND NUMBER PLANTS	REMARKS
101	1EG($S_f S_1$) Bud selfing	S_1S_1	+44 −22	53+ with Number 28 pollen, 1 ♂ sterile
102	1EG($S_f S_1$) Normal selfing	S_1S_1	+10 −3	..
107	1EK($S_f S_1$) Normal selfing	S_1S_1	+9 −3	1 ♂ sterile
110	2EK($S_f S_1$) Bud selfing	S_1S_1	+24 −8	1 ♂ sterile
111	2EK($S_f S_1$) Normal selfing	S_1S_1	+2 −0	..
116	1EO($S_f S_3$) Normal selfing	S_2S_3	+11 −8	..
118	2EO($S_f S_3$) Normal selfing	S_2S_3	+21 −4 ♂ st.	Omitted, 6 ♂ sterile, 1 deficient pollen.
119	2EO($S_f S_3$) Bud selfing	S_2S_3	+52 −7	1♂ sterile, + to S_2S_3
129	1EP($S_f S_3$) Bud Selfing	S_2S_4	+36 −21	Omitted, 1 ♂ sterile, 2 deficient pollen.
130	1EP($S_f S_3$) Normal selfing	S_2S_3	+18 −1	The − plant had deficient pollen.

Note: All plants tested were self-fertile if pollen was produced. No plants had the typical S_2S_3 habit of growth.

ever, if, in addition to the *S* factors and the cytoplasmic factor, another series of allelomorphs located in another pair of chromosomes is postulated. The assumption is made that there are three of these allelomorphs, A_1, A_2, and A_3.

The effects of these factors are as follows: (1) A_1 has no effect in modifying the action of S_1, but tends *slightly* to nullify the effect of S_2 and of S_3, thus permitting some male gametes carrying these factors to function in incompatible matings. (2) A_2 has no effect in modifying the action of S_1, but tends slightly to nullify the effect of S_3 and almost completely to nullify the effect of S_2. It also shows a lethal action on the combination S_1S_3. (3) A_3 has the same action as A_2, except that it shows no lethal action on the combination S_1S_3.

It is assumed, then, that the constitutions of the four plants used in making the original crosses are:

N. Langsdorffii 1 = $S_fS_fA_1A_2$
N. Langsdorffii 3 = $S_fS_fA_1A_3$
N. Sanderæ 29FA = S_1S_1aa
N. Sanderæ 75L = S_3S_3aa

Four combinations were made, which should give eight F_1 classes; but since only eleven F_1 plants were tested, it would not be odd if some of these classes did not appear. The four combinations are: Type I, *N. Langsdorffii* 1×*N. Sanderæ* 29FA (Family EG); Type II, *N. Langsdorffii* 1×*N. Sanderæ* 75L_1 (Family EO); Type III, *N. Langsdorffii* 3×*N. Sanderæ* 29FA (Family EK); and Type IV, *N. Langsdorffii* 3×*N. Sanderæ* 75L_1 (Family EP).

Substituting the formulae given above, these matings produced the following families with the constitutions identified provisionally:

Type I. $S_fS_fA_1A_2 \times S_1S_1aa$ gave $S_1S_fA_1a$ (Plant 2EG) and $S_1S_fA_2a$ (Plant 1EG).

Type II. $S_fS_fA_1A_2 \times S_3S_3aa$ gave $S_3S_fA_1a$ (Plants 1EO, 3EO, 6EO, 7EO) and $S_3S_fA_2a$ (Plant 2EO).

Type III. $S_fS_fA_1A_3 \times S_1S_1aa$ gave $S_1S_fA_1a$ (Plant 1EK) and $S_1S_fA_3a$ (Plant 2EK).

Type IV. $S_fS_fA_1A_3 \times S_3S_3aa$ gave $S_3S_fA_1a$ (Plant 1EP) and $S_3S_fA_3a$ (Plant 2EP).

We have to consider, therefore, the behavior of six groups: (1) $S_1S_fA_1a$, consisting of plants 2EG and 1EK; (2) $S_1S_fA_2a$, consisting of plant 1EG; (3) $S_1S_fA_3a$, consisting of plant 2EK; (4) $S_3S_fA_1a$, consisting of plants 1EO, 3EO, 6EO, 7EO, and 1EP; (5) $S_3S_fA_2a$, consisting of plant 2EO; and (6) $S_3S_fA_3a$, consisting of plant 2EP.

Behavior of $S_1S_fA_1a$ plants

When F_1 plants 2EG and 1EK were crossed with the *Sanderæ* plant $45L_1$ which carried S_2S_2, 55 self-fertile plants and 49 male-sterile plants were obtained. The ratios in the individual families were 23:26 and 32:23 respectively. The self-fertile plants were fertile to pollen of S_1S_1 and S_1S_2 plants without exception. There was a slight tendency for S_2 pollen to function on these S_fS_2 plants, however, for there were 3 positive reactions out of 28 plants tested with an S_2S_2 plant. When this same plant was used on 46 male steriles, only one positive reaction was obtained. When the male steriles (S_1S_2) were tested with S_1S_2 pollen, there were 5 plus tests out of 26 and 4 plus tests out of 23 in the two populations.

In the reciprocal crosses when the *Sanderæ* plant $45L_1$ was crossed with 2EG and 1EK, 56 S_2S_f plants and 55 S_2S_1 plants were obtained. The individual ratios were 29:36 and 27:19. These results appear to show that there was no selective functioning of S_f pollen when in competition with S_1 pollen. No tests were made with S_2S_2 pollen on the plants of the population obtained from the backcross with 2EG, but in the population resulting from the backcross with 1EK there were 4 plus reactions out of 16 plants tested. Tested with S_1S_2 pollen the S_1S_2 plants of these families showed 5 plus reactions in 36 and 3 plus reactions in 19 respectively. Thus there was a consistent tendency for S_1S_2 plants to react plus with S_1S_2 pollen in about 16 percent of all cases tested, though the plants showed no tendency to react plus with S_1S_1 pollen.

Behavior of $S_1S_fA_2a$ plants

The constitution $S_1S_fA_2a$ has been assigned to but one F_1 plant, 1EG. This plant when crossed with the S_2S_2 *Sanderæ* plant 30EA yielded a population consisting of 27 self-fertile plants and 32 male-sterile plants. The self-fertile plants reacted plus to S_2S_2 pollen in 11 out of 19 tests, or 58 percent. The male-sterile plants gave plus reactions to this same S_2S_2 plant in 19 out of 27 trials, or 70 percent. The male steriles (S_1S_2) when tested with S_1S_2 pollen gave plus reactions in 23 out of 30 tests (77 percent), though all showed minus reactions to S_1S_1 pollen.

Unfortunately, it was impossible to test the direct reciprocal of this cross; a sufficient number of usable flowers of both plants were not always available. Instead, the S_2S_2 *Sanderæ* plant $45L_1$ was crossed with pollen from 1EG. On the basis of plus and minus reactions to S_1S_1 pollen, 41 S_2S_f plants and 20 S_2S_1 plants were obtained. In this case, therefore, it would seem that pollen bearing the gene S_f grew faster than pollen bearing the

gene S_1. Obviously, all of the plants of this population would contain the factor S_2; yet 6 plants out of 30 of the S_2S_f plants and 3 plants out of 18 of the S_2S_1 plants reacted plus to S_2S_2 pollen. And 10 out of 20 of the S_1S_2 plants tested reacted plus to S_1S_2 pollen, though none reacted plus to S_1S_1 pollen. There was also evidence of S_2 pollen functioning in the S_1S_2 plants from the self-pollination tests. Of the 20 plants of this constitution 7 were self-fertile.

It is clear, therefore, that when $S_1S_fA_2a$ plants are crossed with S_2S_2aa plants, the plants of the resulting population permit S_2 pollen to function from 3 to 5 times as frequently as do plants of the populations produced when $S_1S_fA_1a$ plants are crossed with S_2S_2aa plants.

Behavior of $S_1S_fA_3a$ plants

The F_1 plant 2EK may be assigned the constitution $S_1S_fA_3a$. It was crossed with an S_1S_1 *Sanderæ* plant 29FA by bud-pollination. Incompatible matings where S_1S_1 pollen is used are usually unsuccessful, but occasionally a few seeds are obtained. The resulting population, in this instance, consisted of 30 self-fertile plants and 15 male-sterile plants. There are no data which enable one to decide why this ratio departs so widely from the one to one ratio expected; but it is probably a chance deviation, since the reciprocal mating shows that $S_1S_1A_3a$ plants are viable. 2EK was also crossed with the S_2S_2 *Sanderæ* plant 30EA and produced a population consisting of 33 self-fertile plants and 27 male-sterile plants. All self-fertile plants were fertile to S_1S_1 pollen, and all male-sterile plants were sterile to S_1S_1 pollen, as was to have been expected. In 15 out of the 27 male-sterile plants, however, S_1S_2 pollen was functional. Additional evidence of the fuctioning of S_2 pollen in these plants is found in 11 plants tested with pollen from an S_2S_2 plant; 9 reacted plus.

2EK pollen was used on the S_1S_1 *Sanderæ* plant 29FA. The population obtained consisted of 9 self-fertile (S_1S_f) plants and 9 self-sterile (S_1S_1) plants, which, as already mentioned, appears to show that both $S_1S_1A_3a$ and S_1S_1aa plants are viable. Two S_2S_2 *Sanderæ* plants, 26EA and 30EA, were also crossed with pollen from 2EK. The resulting populations hardly appear to be random samples of the same type of genetic constitution; yet this is probably the case since in other crosses 26EA and 30EA behaved similarly. The possibility is not excluded, however, that these two plants contain different sterility modifiers. In the first population (115a) there were 15 plants supposedly S_2S_f and 9 plants supposedly S_2S_1; in the second population (115b) the numbers were 4 and 9 respectively. Adding the two families together, we obtain 19 plants of one class and 18

plants of the other. The 4 self-fertile plants of type S_2S_1, and the 3 plants fertile to S_1S_2 pollen out of 9 tested, however, all belong to family 115a.

Behavior of $S_3S_fA_1a$ plants

The plants of this group are 1EO, 3EO, 6EO, 7EO, and 1EP. But one plant, 1EO, was bud-pollinated with pollen from an S_3S_3 *Sanderæ* plant (1DD), and, as in the case with 2EK pollinated with 29FA, there was a deficiency of male steriles. The ratio was 29 to 14. Unfortunately, no reciprocal mating was made, so it is unknown whether or not $S_3S_3A_1a$ plants are viable. Plant 3EO behaves somewhat differently from the others. The ratio of 30 self-fertile plants to 18 male-sterile plants shows a wide departure from a 1 to 1 ratio; 16 of these 18 male steriles were fertile to S_1S_2 pollen, and 8 out of 13 male steriles tested were fertile to S_2S_2 pollen. These facts lead one to suspect that S_3S_2aa plants in conjunction with cytoplasm from *N. Langsdorffii* are not viable; but if this were true, then there would seem to be no reason why similar plants from other matings should be viable.

The total number of self-fertile plants was 125; the total number of male-sterile plants was 100. Of the male-steriles, 84 were tested with S_2S_3 pollen; 46 reacted plus, 38 reacted minus. Male steriles of 3 families were also tested with S_2S_2 pollen. Of 52 plants tested, 12 reacted plus and 40 reacted minus.

The four reciprocals reported were with pollen from plants 3EO, 6EO, 7EO, and 1EP. They were true reciprocals. The S_2S_2 *Sanderæ* plants used were 30EA in the first case and $45L_1$ in the other three cases. The total number of S_2S_f plants was 163 while the total number of S_2S_3 plants was 68, which seems to indicate that S_3 pollen in competition with S_f pollen did not function so frequently. Of the 68 S_2S_3 plants, 16 were self-fertile, which was presumably due to the functioning of S_2 pollen. Forty-four of these plants were tested with S_2S_3 pollen, and 5 were fertile to it. Four out of 39 of these plants were fertile to S_2S_2 pollen. It should be noted, however, that 36 of 99 S_2S_f plants tested were fertile to S_2S_2 pollen. Thus S_2S_f plants allowed S_2 pollen to function much more frequently than did S_2S_3 plants.

Behavior of $S_3S_fA_2a$ plants

Plant 2EO has this formula. 2EO crossed with an S_1S_1 *Sanderæ* plant 29FA gave a population of 30 plants, all of which were self-fertile. The fact that no male steriles appeared is doubtless to be interpreted on the supposition that these plants are not viable in cytoplasm coming mainly

from *N. Langsdorffii*. 2EO was also crossed with two S_2S_2 *Sanderæ* plants, 26EA and 30EA. Eighteen self-fertiles and 16 male steriles were obtained. Nine out of 15 male steriles were fertile to S_3S_3 pollen, apparently a different behavior from that exhibited by $S_3S_fA_1a$ plants where only one case of fertility to S_3S_3 pollen was found in 22 plants tested. Seven out of 12 male steriles were fertile to S_2S_3 pollen.

The reciprocal to family 121a, where the S_2S_2 *Sanderæ* plant 26EA was crossed with plant 2EO, yielded 41 self-fertile S_2S_f plants and only 6 self-sterile S_2S_3 plants, 3 of the latter being fertile to S_3S_3 pollen.

Behavior of $S_3S_fA_3a$ plants

This formula was assigned to plant 2EP. When 2EP was crossed with the S_2S_2 *Sanderæ* plant 30EA, the resulting population consisted of 32 self-fertile plants and 30 male-sterile plants. Twenty-three of 24 male steriles tested were fertile to S_2S_3 pollen, showing that here S_2 pollen grows freely. In tests with 25 of these plants with S_2S_2 pollen, 20 reacted plus. This behavior was certainly very different from the plants belonging to the group designated $S_3S_fA_1a$.

The reciprocal of this mating, S_2S_2 *Sanderæ* 30EA$\times$2EP, produced 32 S_2S_f plants and 24 S_2S_3 plants. Twelve of the 24 S_2S_3 plants were self-fertile and 14 were fertile to pollen of S_2S_3, though none was fertile to S_3S_3 pollen.

It is clear from these data that there is definite consistent evidence of a differential action of two factors independent of the S factors in inheritance—let us say A_1 and A_2—which modify the action of the S factors under consideration. A_2 almost nullifies the action of S_2, and tends slightly to nullify the action of S_3 though it has no effect on S_1. A_1, though also having no effect on S_1, has a very slight tendency to nullify the effect of S_2 and S_3.

It is not so certain that there is a differential effect which requires one to postulate a third allelomorph, A_3. A_3 may perhaps be considered to be too similar to A_2 in its action. I can not maintain that there is critical evidence of the existence of A_3; yet long experience with the self-sterility problem has convinced me that even slight consistent differences in behavior are to be attributed to genetic factors. I can only say, therefore, that when all the facts are taken into consideration, it is reasonable to postulate all three factors, for there is a tenuous thread of difference in the behavior of families which have been designated as containing A_2 and the families which have been designated as containing A_3. The main point, however, is the proof of independent factors which modify the action of certain of the S

genes. The question of how many of these allelomorphs exist is not important.

The cytoplasmic factor

Further information on the effect of the factor carried in the cytoplasm of *N. Langsdorffii* is brought together in table 4.

A male-sterile plant 121–2, coming from F_1 2EO (*Langsdorffii* $1 \times 75L_1$ S_3S_3) $\times$ *Sanderæ* 26EA (S_2S_2), was crossed with *Sanderæ* S_3S_3. The 37 plants obtained were all male sterile. The female plant should be S_2S_3, of course, and should be incompatible with S_3S_3 pollen. The cross was easy to make, however, probably from the action of the factor A_2.

TABLE 4

Inheritance of male sterility.

CROSS	RESULT		
	SELF FERTILE	SELF STERILE	♂ STERILE
121–2 (2EO×26EA) ♂ sterile×*Sanderæ* S_2S_3	0	0	37
112–22 (2EK×29FA) ♂ sterile×*Sanderæ* S_2S_2	0	0	36
118–16 (2EO selfed) ♂ sterile×*Sanderæ* S_3S_3	0	0	25
130–14 (1EP selfed) ♂ sterile×*Langsdorffii*	40	0	0
120–1 (2EO×29FA) self-fertile×*Sanderæ* S_2S_3	32	0	0
119–8 (2EO selfed bud) self-fertile×*Sanderæ* S_3S_3	36	0	0
130–18 (1EP selfed) self-sterile×*Sanderæ* S_2S_3	12	0	23
130–12 (1EP selfed) self-sterile×*Sanderæ* S_3S_3	21	4	11
125–1 (6EO×45L$_1$) self-fertile×*Sanderæ* S_2S_3	26		14

A second cross of similar character was obtained by pollinating male sterile 118–16, coming from F_1 2EO selfed, with pollen from *Sanderæ* S_3S_3. Again all the plants, 25 in number, were male sterile. In this family the plants showing typical S_3S_3 habit of growth were recorded. There were 11 plants having this characteristic.

A third male sterile 112–22, coming from F_1 2EK (*Langsdorffii* $3 \times 29FA$ S_1S_1) $\times$ *Sanderæ* 29FA (S_1S_1), was crossed with *Sanderæ* S_2S_2. As all of the male steriles from the family of the mother should be S_1S_1, then all plants resulting from the cross should be S_1S_2. All of the 36 plants obtained were male sterile; but 8 of them were fertile to S_2S_2 pollen. In other words, S_2 pollen again functioned rather frequently, presumably owing to the influence of factor A_2.

The reciprocal tests of the plants of the F_1 generation of the crosses between *N. Langsdorffii* and *N. Sanderæ* showed clearly that the factor causing male sterility in plants carrying two *S* factors other than S_f is a cytoplasmic factor. The results from the three crosses just described confirm this idea. In addition, a cross was made between a male sterile 130–14, coming from F_1 2EP selfed, and a plant of *N. Langsdorffii*. Bringing in *Langsdorffii* genes with *Langsdorffii* cytoplasm yielded no male steriles. The 40 plants obtained were all self-fertile.

It will be remembered that F_1 2EO ($S_3S_fA_2a$) crossed with 29FA (S_1S_1aa) produced no male steriles. This result was interpreted as a lethal effect on S_1S_3 due to the presence of A_2. There seems to be a similar lethal effect on S_3S_3. This conclusion may be drawn from the fact that two self-fertile plants, one from F_1 2EO×29FA (S_1S_1) and the other from 2EO selfed in the bud, produced only self-fertile plants when crossed with *Sanderæ* S_3S_3.

In other cases, as shown by table 4, both self-sterile and self-fertile plants crossed with *Sanderæ* plants gave self-fertiles and male-steriles just as they did in the previous generation.

The combination "N. Sanderæ×N. Langsdorffii"

Very little work was done with *Sanderæ-Langsdorffii* hybrids where *N. Langsdorffii* was used as the pollen parent; but a sufficient number of observations were made to show that male sterility is uncommon in such combinations. On the other hand, this type of cross appeared to yield more F_2 plants that might be classed as self-steriles than did the reciprocal. Perhaps the action of the factors at the *A* locus was more efficient.

A *Sanderæ* S_2S_2 plant 97–15 was crossed with *N. Langsdorffii*. Twenty F_1 plants were raised. All were self-fertile and were rather easily backcrossed with pollen of the S_2S_2 mother plant, indicating unexpectedly high fertility to S_2.

Plant 301–9 of the F_1 generation was crossed both ways with S_2S_2 plant 97–15 which had entered into the combination.

When the S_2S_2 *Sanderæ* plant was used as female, 97–15×301–9, the backcross generation consisted of 12 normal self-fertiles, 1 male sterile, and 5 plants with deficient pollen.

When the F_1 plant was used as female, 301–9×97–15, no male steriles were produced. Sixteen plants were raised. All were normal self-fertiles. This F_1 plant was also crossed with the pollen from an S_4S_4 plant 97–17. Twelve plants were raised, all of which had normal pollen development and were self-fertile. Thus there was no tendency for the F_1 cytoplasm, which came largely from *N. Sanderæ*, to react badly with *Sanderæ* genes brought

in by the pollen; but there was a tendency for the F_1 (*Sanderæ*) cytoplasm to react badly with the *Langsdorffii* genes. The data are too few for one to say whether self-fertiles and male steriles are produced in equal numbers in the combination (*N. Sanderæ*×*N. Langsdorffii*)×*N. Langsdorffii*; but it seems reasonable to conclude that the type of male sterile found is not so extreme as that which appeared in the backcrosses of the reciprocal F_1 plants.

When the F_1 plant 301–9 was selfed, the resulting progeny consisting of 21 plants fell into the following classes: 12 self-fertiles, 4 self-steriles having some functional pollen, and 5 questionable plants having very few, if any, seemingly perfect pollen grains. In the reciprocal F_2 generation, 314 plants were produced of which only 18 had deficient pollen. In the present case, there were at least 4 self-steriles with deficient pollen out of 16 plants, and, if one includes the questionable plants, there were 9 self-sterile and deficient pollen plants out of 21 plants.

The tests on this type of F_1 were somewhat crude and unsystematic. In themselves they are insufficient to establish behavior norms; yet they lead one to believe that the study of a random sample of F_1, and later, generations coming from different combinations of *Sanderæ* and *Langsdorffii* plants would uncover F_2 segregations of self-fertiles and self-steriles (or male steriles) in a great variety of ratios due to the various types of action of the *S* factors themselves and of the sundry subsidiary factors located at the *A* locus or at other loci.

The inheritance of certain color factors

Corolla color due to the interaction of factors *C* and *P*

SACHS-SKALIŃSKA (1922) has reported that eight genes control the inheritance of corolla color other than green, while two or more genes are effective in producing the various types of green. I have accumulated evidence that there are two color intensifiers and two factors for pigment extension which are probably the same as those which Madame SACHS-SKALIŃSKA has described. BRIEGER and MANGELSDORF (1926) have also described a recessive factor for ivory (*ii*) and a basic color factor *C*. Madame SACHS-SKALIŃSKA discovered a similar factor which produces no color alone but acts in conjunction with a factor *F* (producing a violet color acting alone) to produce a reddish-magenta. The factors *C* and *P*, which I wish to discuss here, may be these same factors, but I will use the nomenclature in which my records have been kept, since their behavior is very different from the *C* and *F* of Madame SACHS-SKALIŃSKA.

BRIEGER and MANGELSDORF found that the factor *C* produces no color in the corolla, though the base of the stem is tinged with purple and the seeds have a dark seed coat. In the absence of *C* (*c*), the base of the stem is green and the seeds have a light tan seed coat. *C* is linked with sterility factors at the *S* locus; and BRIEGER and MANGELSDORF determined that crossing over was 24.5 percent and 16.2 percent in microsporogenesis and 18.2 percent and 14.5 percent in megasporogenesis in two families L_1 and R_1, and 13.6 percent in microsporogenesis in a third family N_1.

I wish to present some further data on this factor *C* together with facts relative to the action of factor *P* which, though producing no visible effect by itself, acts with *C* to produce a purplish-magenta color.

The color of the corollas of the *Langsdorffii* plants of P_1 was green (S_fC S_fCpp), while the color of the corollas of the *Sanderæ* plants of P_1 was white (S_1cS_1cPP for 29FA and S_3cS_3cPP for $75L_1$). The corollas of the F_1 plants were purplish-magenta. The *Sanderæ* plants used in the backcrosses had the following constitutions: 26EA was S_2cS_2cPp, 30EA was S_2cS_2cPp, $45L_1$ was S_2CS_2CPP, and 1DD was S_3cS_3CPP. Obviously, crossover percentages can be calculated from some of the backcrosses. The most reliable of these calculations are those for macrosporogenesis, since there is no question but that the male-sterile plants are the ones which have two *S* factors other than S_f in their constitution. The results are found in table 5.

TABLE 5

Crossing over in macrosporogenesis between C and S_n.

FAMILY	PARENTAGE	+		♂ STERILE		PERCENT CROSSOVERS	APPROXIMATE RATIO
		C	*c*	*C*	*c*		
103	1EG×30EA	22	5	8	21	23	3:1
133	2EP×30EA	25	7	8	20	25	3:1
120	2EO×29FA	28	2	..	..	7	6:1
121a	2EO×26EA	11	1	3	8	17	..
121b	2EO×30EA	5	1	1	4	18	..
112	2EK×29FA	23	7	2	9	22	8:3
114	2EK×30EA	27	10	8	21	27	..
117	1EO×1DD	24	4	..	..	14	5:1
123	3EO×30EA	26	3	3	15	12	6:1

Note: The approximate ratios of the last column were used in the calculations.

The number of plants involved is very small, and for this reason one can not have a great deal of confidence in the calculation for the individual family; but the variability of the crossover ratio is high, just as has been found in all other such studies. As GOWEN has remarked, this fact seems to

show that the mechanism which causes crossing over is not so precise as that behind most physiological processes.

There is one other point here to which I wish to direct attention. Family 120 contained no male steriles. The conclusion was drawn that male steriles had been produced but were not viable. It might be claimed that the 30 self-fertiles obtained in this population were really of two classes, self-fertile and self-sterile, and that some unknown factor made them appear to be the same. The crossover data show that this was not the case. There were 28 plants containing *C* and 2 plants containing *c*. If the male-sterile class were present—in disguise, so to speak—the ratio of *C* plants to *c* plants would be approximately equal.

The crossover percentage for plant 2EK was approximately the same in microsporogenesis as in macrosporogenesis. The crossover percentages in the three other families for which they are available in microsporogenesis show clearly that we were justified in placing some of the plants which gave seed after selfing in the self-sterile group on the basis of their incompatibility with certain test plants. The self-fertility found was attributed, it will be remembered, to the presence of the modifying factors at the *A* locus.

When the plants of these three families are segregated on the sole criterion of self-fertility or self-sterility, the crossover results are as follows:
Family 134, 30EA×2EP gave self-fertile 16*C* and 37*c*, self-sterile 2*C* and 10*c*.
Family 122, 26EA×2EO gave self-fertile 21*C* and 19*c*, self-sterile 0*C* and 8*c*.
Family 124, 26EA×3EO gave self-fertile 12*C* and 21*c*, self-sterile 3*C* and 7*c*.
It is obvious, since the self-fertile plants showed more crossovers than non-crossovers, that some of the plants classified as self-fertile are really self-sterile. On the basis of classification by reaction to the testers the results are:
Family 134, self-fertile 15*C* and 7*c*, self-sterile 6*C* and 20*c*.
Family 122, self-fertile 17*C* and 10*c*, self-sterile 1*C* and 5*c*.
Family 124, self-fertile 11*C* and 8*c*, self-sterile 5*C* and 19*c*.
The numbers do not check because only a part of the plants of each family could be segregated on the basis of the tests. As they stand, the figures still show a marked excess of crossover gametes over expectancy; but they are more nearly normal than they are in the first classification.

The inheritance of the *P* factor

The *P* factor is independent of the *S* factors and the *C* factors. The class expectancies for purple and non-purple can be calculated on the basis of

the crossover percentages for *C* and *S* that were found. When this was done, the fit was very good on the backcrosses where the F_1 plants were used as females. Two sample calculations are given below in table 6, using a 3:1 crossover ratio. Both parents were heterozygous for *P*.

TABLE 6

Inheritance of the P factor.

FAMILY NUMBER	TYPE	FOUND	EXPECTED	RATIO
Number 103	+ *P*	13	14	9
	+ ♀	12	11	7
	♂ sterile *P*	5	5	3
	♂ sterile ♀	23	23	13
Number 133	+ *P*	18	18	9
	+ ♀	14	14	7
	♂ sterile *P*	4	5	3
	♂ sterile ♀	24	23	13

In the reciprocal crosses, where the *Sanderæ* plants were crossed with the F_1 plants, using the latter as males, the data for purple and non-purple are confusing. There is a variable excess of non-purples too great to be attributed to chance. In fact, very few purples were produced. Evidently the *A* factors inhibit the development of color when the cytoplasm derives largely from *N. Sanderæ*; but whether there is a differential effect to be ascribed to the two (or three) *A* factors, I can not say.

Inheritance of anther color and pollen color

Dark anthers are produced ordinarily by the interaction of independent factors *C* and *P*, or by the interaction of *C* with another independent factor *D*. Combinations *CP*, *CD*, and *CPD* are characterized by dark anthers. Plants having any of these factors alone have light anthers. The combination *S/cDBP*, however, behaves queerly. With *Langsdorffii* cytoplasm, plants of this constitution have dark anthers; with *Sanderæ* cytoplasm, they have light anthers.

Blue pollen is due to the interaction of three independent complementary factors *CDB*. The factor *D* (with *C*) apparently does not produce blue pollen.

Here again the cytoplasm exerts its influence. In the crosses between the F_1 plants and the *Sanderæ* plants used as males there is a normal state of affairs. This can be seen by comparing the observed ratios with the expected ratios in table 7. The calculations were made by using crossover

TABLE 7

Inheritance of anther color and pollen color $F_1 \times Sanderae$.

FAMILY NUMBER	CROSS	DARK ANTHER BLUE POLLEN		DARK ANTHER WHITE POLLEN		LIGHT ANTHER WHITE POLLEN	
		OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED
103	$S_1cS_fCPpDdBb \times S_2cS_2cPpddbb$	8	5	13	14	4	6
112	$S_1cS_fCPpDdBb \times S_1cS_1cPPddbb$	10	11	13	15	?	4
114	$S_1cS_fCPpDdBb \times S_2cS_2cPpddbb$	7	7	20	20	10	10
121b	$S_3cS_fCPpDdBb \times S_2cS_2cPpddbb$	0	1	5	4	1	1
123	$S_3cS_fCPpDdBb \times S_2cS_2cPpddbb$	6	7	23	18	2	6
133	$S_3cS_fCPpDdBb \times S_3cS_2cPpddbb$	7	6	18	18	7	8
117	$S_3cS_fCPpDdBb \times S_3cS_3cPPddbb$	5	6	20	20	3	1
Total		43	43	112	109	34	36

ratios of 3:1 for families 103 and 133, of 6:1 for families 121b and 123, of 8:3 for families 112 and 114, and 5:1 for family 117, these being the ratios which seemed most probable when all the data were considered. The fit is excellent. Even if a rough average crossover value of 4:1 is used for all families, the fit is satisfactory. On the other hand, when the data from the reciprocal crosses are examined—*Sanderæ* crossed with F_1 plants used as males—there is again some confusion. In certain combinations where S_f and one or perhaps two of the A factors are united, *and the cytoplasm comes from N. Sanderæ*, there is a great excess of light anthers and blue pollen. Thus the combination S_f and A_3 (especially) with *Sanderæ* cytoplasm is unfavorable for the development of dark anthers and blue pollen.

Doctor EDGAR ANDERSON permits me to say that the data on *alata-Langsdorffii* crosses, gathered by him and by Miss DE WINTON, show evidence of a factor for pollen color that lies in the same chromosome as the *C* and the *S* factors. I suspect that the data which I have collected exhibit the same phenomenon. At least, such an assumption is compatible with the facts observed in several families. But the data reported in table 7 can not show the presence of such a factor in any conclusive manner because the male steriles produce no pollen; and the backcrosses where no male steriles appear are complicated by the action of the A factors.

The inheritance of green versus non-green corolla

Green in the corolla of *N. Langsdorffii* is controlled by a factor *G* which is absent from *N. Sanderæ*. So much is clear from table 8, showing the results after backcrossing various F_1 plants to *N. Sanderæ*. The first two families may be simple 1:1 ratios due to the union *Gg* with *gg*. But it is possible that a complication exists here. Family 103 is 1EG ($S_1cS_fCA_2aGg$) × 30EA (S_2cS_2caagg). Family 112 is 2EK ($S_1cS_fCA_3aGg$) × 29FA (S_1cS_1caagg). The excess of green plants, though perhaps negligible, may be due to the fact that combinations $S_2cS_fcA_2agg$ and $S_1cS_fcA_3agg$ are favorable to the production of chlorophyll when with *Langsdorffii* cytoplasm. The tendency appears to be reversed in the reciprocal crosses. Plant 29FA crossed with 2EK gave 0 green plus, 9 non-green plus, 5 green minus, and 5 non-green minus. Plants 26EA and 30EA crossed with 2EK gave 3 green plus, 14 non-green plus, 5 green minus, and 5 non-green minus. It is suggested, therefore, that plants 26EA, 30EA, and 2EK are heterozygous for a factor *Zz* which is favorable to the production of chlorophyll when S_f is present with *Langsdorffii* cytoplasm and unfavorable to the production of chlorophyll when double sterility factors are present in *Sanderæ* cytoplasm.

TABLE 8

Inheritance of green versus non-green corolla.

FAMILY	+		♂ STERILE	
	G	NON-*G*	*G*	NON-*G*
103	14	11	14	14
112	17	13	6	5
114	20	17	9	20
120	7	23	..	..
121	3	27	6	21
123	9	22	12	6
133	13	19	23	5

Plant 2EO evidently has a different constitution, as it gives a marked excess of non-green plants in crosses where 29FA, 26EA, and 30FA were used as males. Plant 26EA was also crossed with pollen from 2EO, and yielded 4 green plus, 4 non-green plus, 16 green minus, and 24 non-green minus. If we rely on the backcrosses shown in table 8, for the reasons given earlier, the results with 2EO can be interpreted by assuming that it is heterozygous for a factor *X*, coming originally from *N. Sanderæ*, that inhibits the action of *G*, but is effective only with S_f and *Langsdorffii* cytoplasm. Plants 3EO and 2EP may possibly have another factor for green, G_rg_r, independent of

G. It acts only in the presence of two *Sanderæ* sterility factors. *Sanderæ* plants 26EA and 30EA would be $Zzxxg_rg_r$. Plant 30EA×2EP gave 30 green plus, 23 non-green plus, 5 green minus, and 3 non-green minus. Plant 30EA×3EO gave 16 green plus and 17 green minus.

The rather complex interpretation of these data set forth here gives an excellent fit. It was designed to fit and naturally does fit. But I do not claim any proof of the precise factorial analysis made. What seems to be more important is the difficulty of conceiving of any analysis which does not postulate that certain combinations behave differently in *Langsdorffii* cytoplasm and in *Sanderæ* cytoplasm. I believe that for this one fact the evidence is good.

SUMMARY

1. *N. Langsdorffii* contains a fertility factor—a factor evoking rapid pollen-tube growth in all combinations—which is allelomorphic to the sterility factors found in *N. Sanderæ*. It is designated S_f.

2. When plants of *N. Sanderæ*, homozygous for known self-sterility factors, are crossed reciprocally with plants of the self-fertile species *N. Langsdorffii*, the F_1 plants S_fS_n are self-fertile.

3. S_fS_n plants, unless they contain modifying factors, are sterile to the pollen of S_nS_n plants and to the S_n pollen of S_nS_z plants. In the absence of other variables, therefore, S_fS_n plants selfed yield F_2 populations consisting of equal numbers of plants belonging to classes S_fS_f and S_fS_n, both of which are self-fertile. There is but one known exception to this rule of behavior for *S* factors. A factor discovered by ANDERSON and DE WINTON in *N. alata* and designated S_F by them has the property of inhibiting the development of S_f pollen tubes. Under optimum conditions it may be expected that some seed would be produced by selfing S_fS_F plants and that both self-fertile and self-sterile offspring would be produced.

4. Plants S_fS_n crossed with plants S_fS_z will produce self-fertile and self-sterile plants in the ratio of 3 to 1. The self-sterile plants will be incompatible with each other in reciprocal crosses.

5. Genes other than *S* factors may exist which will so modify the effects of the primary *S* factors that a plant $S_fS_nX_1X_2$ selfed could produce both self-fertile and self-sterile offspring (see KAKIZAKI 1930). It is not improbable, therefore, that the results which I reported in 1919 for crosses between *N. Langsdorffii* and *N. Sanderæ*—where selfed F_1 plants gave both self-fertile and self-sterile plants in the F_2 generation—were correct. The constitutions of the plants used in these earlier experiments were unknown. Many modifying factors may have been present.

6. The effects of three modifying factors independent of the *S* locus—A_1, A_2, and A_3—are described. These three factors have no effect on the action of S_1, and but a slight tendency to nullify the action of S_3 in incompatible matings. A_1 tends *slightly* to nullify the action of S_2 in incompatible matings. A_2 tends *markedly* to nullify the action of S_2 in incompatible matings and appears to have a lethal action on the zygote which contains S_1S_3. A_3, the existence of which is more questionable, appears to have no lethal effect on zygotes which contain S_1S_3, though otherwise it behaves like A_2.

7. *N. Sanderæ*, in so far as it was tested, seems to be homozygous for a factor *aa* at the *A* locus. The *N. Sanderæ* plants used have been closely bred for about ten generations and should be homozygous for most factors.

8. Plants carrying two *S* factors other than S_f were male sterile in the presence of cytoplasm derived largely from *N. Langsdorffii*. They produced normal pollen and were self-sterile (apart from the effect of the *A* factors) when in the presence of cytoplasm derived largely from *N. Sanderæ*.

9. The inheritance of certain factors *C* and *P* affecting corolla color is discussed. *C* lies in the same chromosome as *S*. The crossover values in crosses between *N. Langsdorffii* and *N. Sanderæ* appear to be of the same order of magnitude as in *N. Sanderæ*—at least in megasporogenesis.

10. Anther color is due to the reaction of factors *C*, *P*, and *D*. *CP*, *CD* and *CDP* give dark anthers. Plants of these constitutions have dark anthers with *Langsdorffii* cytoplasm, and light anthers with *Sanderæ* cytoplasm.

11. Blue pollen is due to three supplementary factors acting in combination, *CDB*. *Sanderæ* cytoplasm, when in combination with S_f and with certain *A* factors (especially A_3), is unfavorable to the development of blue pollen.

12. Green in the corolla is due, primarily, to a dominant factor *G* in *N. Langsdorffii*. There is evidence that certain *N. Sanderæ* plants contain a factor *Z* which is favorable to the production of chlorophyll in the corolla when S_f is present with *N. Langsdorffii* cytoplasm and unfavorable to the production of chlorophyll when two *S* factors other than S_f are present and the cytoplasm derives largely from *N. Sanderæ*. A third factor *X*, coming from *N. Sanderæ*, appears to inhibit the action of *G*, but is effective only with S_f and in *N. Langsdorffii* cytoplasm. A fourth factor for green, G_r, may exist. G_r appears to act only when two *N. Sanderæ* sterility factors other than S_f are present with *N. Sanderæ* cytoplasm.

13. Certain combinations of factors, therefore, produce different phenotypic effects depending on the derivation of the cytoplasm.

14. Certain combinations of factors are viable or non-viable depending on the derivation of the cytoplasm.

15. *N. Sanderæ* is treated as a species. It is a horticultural combination of *N. Forgetiana* and *N. alata*, and behaves as do the parental species. The distributions of the wild species *N. Forgetiana*, *N. alata*, and *N. Langsdorffii* are not well known, and one can not say whether they overlap or not. But *N. Langsdorffii* is certainly distinct from the other two on any basis except sterility in hybrid combinations. Nevertheless, though the *distribution* of genes is normal in the hybrid combination *N. Langsdorffii* × *N. Sanderæ*, the two species have come to differ in such a way that many combinations of factors which are successful in the pure species are unsuccessful in the cytoplasm of the other species.

ACKNOWLEDGMENT

I am very grateful to Doctor S. H. YARNELL for his effective help in gathering the data reported here and in interpreting their meaning.

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THE INHERITANCE OF RESISTANCE TO *SALMONELLA AERTRYCKE* IN VARIOUS STRAINS OF MICE[1]

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INTRODUCTION

Disease resistance in animals is a field in which there has been relatively little investigation from the genetic point of view. With the development of more precise methods for laboratory control of diseases and of heredity, more critical experiments from the genetic standpoint are demanded of the animal pathologist.

The experiments reported in this paper were planned to investigate the factors involved in reaction differences among various laboratory strains of mice when subjected to a controlled infection with a specific bacterial disease. Selection for resistance was the major object of the investigation using the survival of progeny as a criterion for the breeding in successive generations. Although this study was concerned exclusively with reactions inherent in mice, precedent for the general plan of investigation was drawn directly from experiments on rats conducted in the Genetics Laboratory by IRWIN (1928, 1929) and on poultry by LAMBERT and KNOX (1928).

The increased resistance among rats to the Danysz bacillus observed by IRWIN over three generations of selection was attributed to complex genetic factors some of which apparently were partially dominant in inheritance. LAMBERT and KNOX, using the reaction in chicks as a basis for selection, also suggested that multiple genetic factors, in part, determine resistance to fowl typhoid. ROBERTS and CARD (1926), likewise working with chicks, showed that resistance to bacillary while diarrhea was definitely influenced by heredity. Preceding this, FRATEUR (1924) had proposed a single factor for determining resistance to avian diphtheria. Among highly inbred lines of guinea pigs, WRIGHT and LEWIS (1921) noted appreciable differences in reaction to tuberculosis.

In reports dealing with bacterial infections of mice, various authors have observed individual as well as strain and stock differences in resistance. TYZZER (1917) and HAGEDOORN-LABRAND and HAGEDOORN (1920) observed that during laboratory epidemics the Japanese waltzing mouse stocks were much less resistant to infections than were other mouse stocks. PRITCHETT (1926) reported distinct differences of reaction between inbred

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strains of mice when subjected to controlled doses of paratyphoid-enteritidis and Type II mouse typhoid (*Bacillus pestis caviae*).

Among the investigations dealing with infections of mice, WEBSTER (1924, 1925) has presented the most clear-cut evidence of heredity as a factor in resistance. He has shown that by selective breeding resistance could be increased or diminished when using paratyphoid-enteritidis as the infective agent.

MATERIALS AND METHODS

The mouse (*Mus musculus*) was selected as a subject for this study because of its wide use in pathological work, its adaptability to laboratory conditions, and its extensive use in hereditary investigations. In 1928, when this study was initiated, the strains available for tests were the Bagg albino, Strong dark-brown, Little dilute-brown, and a commercial strain designated as (Sch) Schwing albino. Later three other lines were acquired, Short-eared, pink-eyed, dilute-brown; English silver; and a white-faced, or piebald strain.

The foundation stocks for the strains came from various sources as follows: Doctor E. C. MACDOWELL of the CARNEGIE INSTITUTION OF WASHINGTON supplied the Bagg albino (Ba) strain in 1924. This strain had been developed by Doctor H. J. BAGG of MEMORIAL HOSPITAL, New York in 1916, and had been inbred by MACDOWELL since 1922. Other stocks provided by MACDOWELL were the Little dilute-brown (Li) strain in 1926; a short-eared, pink-eyed, dilute-brown (Pbr), and an English silver (Sil) strain early in 1929. The Li strain was developed by Doctor C. C. LITTLE while at the UNIVERSITY OF MAINE; the Pbr strain was isolated by Doctor CLARA J. LYNCH of the ROCKEFELLER INSTITUTE; and the Sil strain was from stock imported from England in 1927 by Doctor L. C. DUNN of COLUMBIA UNIVERSITY. The Strong dark-brown (Str) strain originally came from Doctor L. C. STRONG of the BUSSEY INSTITUTION in 1926. The Schwing albino (Sch) strain came from Mr. ED SCHWING, a dealer in Harrisburg, Pennsylvania, in 1927. The white-faced (Wf) strain came from BUSSEY INSTITUTION stock in 1930 through courtesy of Doctor M. R. IRWIN, now of the UNIVERSITY OF WISCONSIN.

All of the strains except the Sch and Sil have been continued by brother-sister or parent-offspring mating. The Sch strain has been carried as a pen-inbred line. Progeny from all of the seven strains have been used in tests as described in the body of this report.

The disease organism used in this study is *Salmonella aertrycke*, a species placed by WELDIN (1927) and by BERGEY (1930, pp. 339–350) in the genus Salmonella of the colon-typhoid group of bacteria. It is well known as

a specific typhoid producer in mice and as a producer of typhoid-like diseases in other laboratory animals. Furthermore, it is extensively associated with food poisoning in man (JORDAN 1931, pp. 144–147, and SAVAGE 1925). A culture was supplied to us in 1926 through the courtesy of Doctor W. W. C. TOPLEY, PUBLIC HEALTH LABORATORY, Manchester, England. TOPLEY and his associates have carried on extensive epidemiological and virulence studies with this organism using the mouse as the host (TOPLEY 1925, LOCKHART 1926, WILSON 1930). A stock of the organism used in the Genetics Laboratory is carried in the American Type Culture Collection, JOHN MCCORMICK INSTITUTE, listed under material supplied by M. R. IRWIN, 1926, as *S. aertrycke*, No. 854—"2188 (Type)."

In our laboratory the organism has been carried by monthly transfers on veal infusion agar slants. For each inoculation an 18 hour culture was suspended in 5 cc physiological salt solution and transferred to a sterile stock bottle. A sample was then drawn from the stock bottle and heated for 20 minutes at 55–60°C to render the organism non-motile. The number of organisms was then determined by use of a Petroff-Hausser bacteria counter, and the original suspension diluted so that the required number of bacteria were contained in 0.25 cc of physiological salt solution.

The method of inoculation in all cases was by intraperitoneal injection, the inoculations being made immediately after the dilution was complete. This procedure was considered as a direct and controllable means of uniform infection with the disease.

At approximately 60 days of age the animals were weighed and taken into the isolation laboratory. After inoculation the animals were checked each morning and mortalities recorded over a period of 21 days.

STANDARDIZATION OF DOSAGE

At the outset of this study it was not known how large a dose could be given to an individual mouse of any given strain and still allow for survival and reproduction of a few individuals within the tested group. Accordingly, at consecutive times when animals were available, doses of various fixed numbers were administered.

A suggestion for the usable range of dosage was drawn from LOCKHART'S (1926) summary of mortalities under various doses of the organism. Following intraperitoneal inoculation with graded doses LOCKHART'S experiments show:

Dose	*Number of mice at risk*	*Percentage mortality at fourteenth day*
10^7	400	90.75
10^5	400	70.00
10^3	400	59.75
10	400	30.75

The results of the various doses used in our laboratory on the Schwing strain are summarized in table 1. Here it is shown that a dose of 2×10^5 organisms or over allows for little survival, and it is of interest to note that none of these survivors produced offspring. Doses of 5×10^4 organisms permitted some survival followed by ability to reproduce. The lowest dose administered, 10^4 organisms, allowed distinctly more survival. However, the survivors of this low dose were not used in these experiments because it was believed that animals surviving the next higher dose would possess more hereditary factors for natural resistance.

TABLE 1

Percentage mortality in Sch mice following inoculation with different numbers of S. aertrycke.

NUMBER ORGANISMS IN DOSE	NUMBER INOCULATED	NUMBER DEAD	PERCENTAGE MORTALITY
1×10^7	64	64	100.0
2×10^6	111	109	98.2
2×10^5	228	219	96.0
5×10^4	538	443	82.3
1×10^4	102	71	69.6

The large number of animals listed as tested under the 5×10^4 dose is an accumulation of groups used throughout the experiment as checks for testing the consistency of the virulence of the organism. The survivors from the earlier tests with this standard dose were the progenitors of succeeding generations of our resistant strain.

The rate of mortality occurring under each of the various doses is presented in figure 1. The death rate in each group is expressed in daily percentage mortalities over the test period of 21 days.

The mortality curve of the highest dose can be interpreted as the result of an acute form of infection. The curves of the 2×10^6 and 2×10^5 doses show few differences. The small number of animals in these two groups living over 14 days can probably be ascribed to their sustaining a sub-acute infection. In the reaction given by the lowest dose, 10^4 organisms, the sharpest rise is before the fourteenth day, but the subsequent flattening of the curve is less marked than under the standard dose.

Another striking feature shown by the curves is the similarity of the incubation period in the doses of 2×10^6, 2×10^5, and 5×10^4 organisms. The first mortality occurs on the third day in all three cases. With the dose of 10^7 organisms fatalities are evident on the first day, while with the lowest dose virtually no mortalities occur until the seventh day.

Another feature of the standard dose may be noted in the proportionate differences between the mortalities of the various groups. These statistics

have been calculated according to FISHER's (1930, p. 84) tests for independence and are given in table 2. The difference between the standard dose (5×10^4) and the one four times as large is well beyond that expected from random variation. The same can be said of the standard dose in relation to the one which is five times smaller.

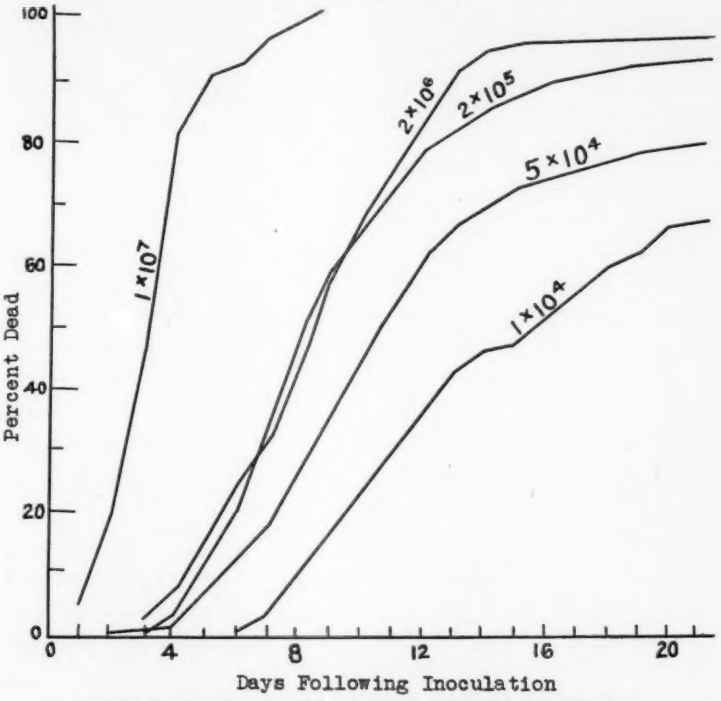

FIGURE 1.—Death rates in Sch mice over a period of 21 days following various doses of *S. aertrycke*.

TABLE 2

Comparison of the differences in percentage mortality resulting from various doses of S. aertrycke.

DOSES COMPARED	DIFFERENCES IN PERCENTAGE MORTALITY	X^2	P
10^7 and 2×10^6	1.8	2.024	0.16
2×10^6 and 2×10^5	2.2	3.424	0.07
2×10^5 and 5×10^4	13.7	26.738	±.01
5×10^4 and 10^4	12.5	8.946	<.01

Simultaneously with the preliminary tests on the Schwing mice, animals from the Bagg albino strain were inoculated with the three lower doses. Likewise Strong dark-brown entered the tests under the 2×10^5 and 5×10^4 doses. The results from these tests are summarized in table 3, and the mortality rates are presented in figure 2.

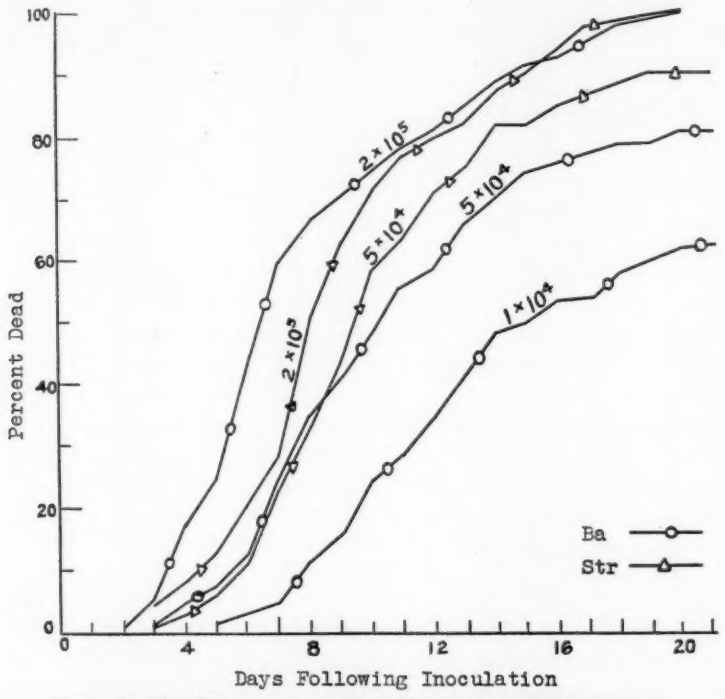

FIGURE 2.—Mortality rate curves in Bagg albino and Strong dark-brown stocks following inoculation with varying doses of *S. aertrycke*.

It should be noted that although the Ba strain is more highly inbred than the Sch strain the mortality in each strain under similar dosage is almost identical (see tables 1 and 3). The reaction of the Str strain is like that of the Ba in the 2×10^5 dose, but with the standard dose the mortality is appreciably higher.

The reactions of these three strains of mice under the various doses were taken as an index of the utility of the 5×10^4 dose as a standard.

TABLE 3

Percentage mortality in Ba and Str strains following various doses of S. aertrycke.

DOSE	STRAIN	NUMBER INOCULATED	NUMBER DEAD	PERCENTAGE MORTALITY
2×10^5	Ba	90	90	100.0
2×10^5	Str	78	78	100.0
$5\times10^{4*}$	Ba	97	78	80.4
$5\times10^{4*}$	Str	54	49	90.7
1×10^4	Ba	56	35	62.5

* Standard dose.

REACTIONS UNDER THE STANDARD DOSE

A statistical method for use when the observations are expressed in percent is illustrated with the data in table 4 and the following formulae. The method, as developed from various statistical considerations by Professsors G. W. SNEDECOR and A. E. BRANDT of the Mathematics Department, IOWA STATE COLLEGE, tests a series of percentage probabilities for homogeneity. In cases of non-homogeneous material, the method may reveal the character of the variation.

TABLE 4

The percentage dead in 13 samples of Sch mice inoculated with the standard dose, 5×10^4 organisms.

NUMBER INOCULATED (I)	NUMBER DEAD (D)	PERCENTAGE MORTALITY (p)
40	34	85.00
39	27	69.23
68	59	86.76
62	51	82.23
76	59	77.63
32	30	93.75
20	16	80.00
66	61	92.42
40	31	77.50
36	27	75.00
27	20	74.07
12	11	91.66
20	17	85.00
538	443	82.34

The sample of 538 Sch mice was tested in sub-samples at thirteen different times. The number inoculated at each time is listed under I, the number dead under D, and the percentage dead under p.

Other symbols used in the formulae are as follows:

$\bar{p}$ = the weighted mean mortality percentage.
$\bar{q} = 100 - \bar{p}$.
N = the number of sub-samples.
n = the average number in the sub-samples.
σ = the weighted standard deviation in percent.
σ_B = the Bernoulli standard deviation.
L = the ratio of $\frac{\sigma}{\sigma B}$ (Lexian ratio).

Then

$$\sigma^2 = \frac{100 \Sigma D\hat{p}}{\Sigma I} - \bar{p}^2 = \frac{100 \times 36745.41}{538} - (92.34)^2 = 50.13$$

$$\sigma_B{}^2 = \frac{\bar{p}\,\bar{q}}{n} = \frac{82.34 \times 17.66}{41.38} = 35.14$$

$$L^2 = \frac{50.13}{35.14} = 1.4265$$

$$L = 1.19$$

When L = 1 a series is interpreted as having a binomial distribution of percentages, the probability of death being constant throughout the sample. With L > 1 a series is said to be supernormal, and is interpreted as due to variation of probability of death from one sub-sample to another. When L < 1 the series is subnormal, the probability varying from individual to individual in each sub-sample, the same series of probabilities being repeated from sub-sample to sub-sample.

The X^2 test for homogeneity furnishes a probability that the variation in percentage dying is due to random sampling. To apply this to the data above:

$$X^2 = NL^2$$
$$= 13 \times 1.4265 = 18.544$$

Then from FISHER's (1930, p. 96) X^2 tables, the above X^2 value is found under n = 12, that is, N − 1, and the corresponding P = 0.10.

This value of P means that a X^2 of 18.544 or larger would be expected from random variation 10 times per hundred in similarly drawn samples. In other words, the variation in mortality percent among the sub-samples in table 4 is well within that attributable to random sampling.

It may be noted that the grouping of the Sch mice into thirteen samples results from routine testing and is entirely arbitrary. A more natural basis for the sub-sample is the litter. Individual records on 25 litters of Sch mice

are available. A comparison of these litters with the whole population and with the three test groups into which they fall is as follows:

	Total number	*Number dead*	*Percentage mortality*	*L*	X^2	*P*
13 test samples	538	443	82.3	1.19	18.54	0.10
25 litters	107	87	81.3	0.97	23.91	0.47
The 25 litters in 3 test groups	107	87	81.3	0.69	1.44	0.49

Here the different classifications show no tendency to disturb the binomial distribution. However, in the Ba mice we have:

	Total tested	*Number dead*	*Percentage mortality*	*L*	X^2	*P*
30 litters	97	78	80.4	1.20	43.80	0.04
In 4 test samples	97	78	80.4	1.03	4.26	0.23

The smallness of the probability and the supernormal Lexian ratio under the litter grouping suggest that there may be significant differences in probability of death from litter to litter in this strain.

A summary of the reactions of the various strains tested with the standard dose is given in table 5.

TABLE 5

Percentage mortality and test for type of dispersion within each of the strains under a standard dose of S. aertrycke (5×10^4 organisms).

STRAIN	NUMBER INOCULATED	NUMBER DEAD	PERCENTAGE DEAD	NUMBER OF LITTERS	L	X^2	P
Sch	534	443	82.3	13*	1.19	18.54	0.10
Ba	97	78	80.4	30	1.20	43.80	0.04
Str	54	49	90.7	19	0.98	18.67	0.48
Li	56	54	96.4	14	0.97	13.23	0.36
Pbr	86	73	84.7	19	1.61	43.62	0.01
Sil	108	108	100.0	..	..	..	..
Wf	71	71	100.0	..	..	..	..

* Number of test samples.

Three of the strains, the Sch, Ba, and Pbr, show approximately the same total mortality. The P of slightly less than 0.01 for the Pbr strain suggests some variation from litter to litter. The Sil and Wf strains show total susceptibility, while the other two strains show a reaction intermediate between the higher and lower figures. In so far as is known, the Wf, Pbr, Li, Str, and Ba strains are more highly inbred than the Sch and Sil strains, but the observed mortality would seem to indicate no difference between the strains on the basis of the degree of inbreeding.

The percentage mortality rates of the seven groups are given in figure 3. There is no slow or rapid reaction characterizing the inbred strains as a group. The extremes of the reaction rates are represented by the Ba, slowest, and the Li, most rapid, among the inbred lines. The less highly inbred strains, the Sil and Sch, also follow the extremes of the mortality rate curves, the Sil high, and the Sch low.

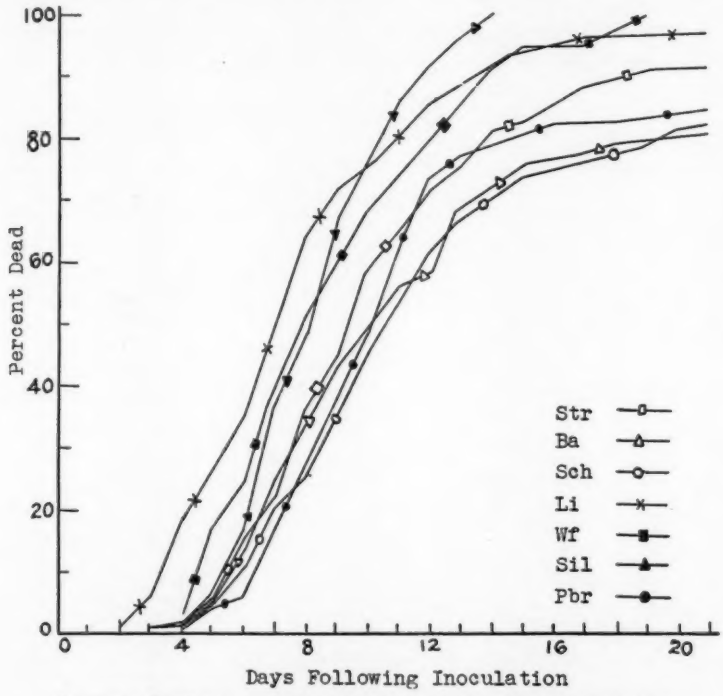

FIGURE 3.—Death rate curves of various strains of mice when inoculated with a standard dose of *S. aertrycke*, 5×10^4 organisms.

VIRULENCE OF THE ORGANISM

In studies employing living bacteria over long periods of time the question of constant virulence is most imperative. It has already been pointed out that a standard dose of 5×10^4 organisms was selected early in the course of this experiment. Throughout a period of three years 1006 mice besides those directly under selection have been tested with this standard

dose; at no test period has the organism failed to produce its quota of fatalities.

That variations do occur in cultures of *S. aertrycke* apparently spontaneously as well as under specific changes of treatment has been shown by LOCKHART (1926) and WILSON (1928, 1930, 1931). However, certain strains of the organism have been observed to maintain a high virulence over many years of culture on laboratory media. Using *S. aertrycke* and other closely allied types of paratyphoid-enteritidis organisms, WEBSTER (1923, 1924, 1925) and PRITCHETT (1926) have observed consistent pathogenicity for the mouse over long periods of time.

In summarizing his specific studies on the virulence of *S. aertrycke* WILSON (1930) states: "It appears probable that the variation in the percentage mortality following the inoculation of the control strain was due not so much to an alteration in its virulence as to fluctuations in the susceptibility of the mice."

The summary of percentage mortalities of the various strains, as given in table 5, shows that the fatalities have been high and uniformly distributed through each strain except in perhaps Pbr and Ba. In the Pbr strain the total mortality has been higher than that of the Ba or Sch strains and the apparent fluctuation from litter to litter within the strain in no way invalidates the conclusion that the organism has maintained a high and fairly consistent virulence.

RESULTS OF SELECTION FOR RESISTANCE

Selection for resistance to the disease organism has been carried on through six generations. The original progenitors for the selected group came from survivors of the Sch strain. Successive selected generations are designated as S_1S_2, etc. A summary of the selected group is given in table 6, along with the reactions of the Sch and Sil strains and a hybrid stock from Sch$\times$Sil. The stock listed as Oc represents progeny from selected resistant animals from the S_1 to S_5 mated with Silvers. Considerations of the results of reciprocal matings are made in the discussions of passive immunity and sex differences.

The S_1 animals are progeny of survivors of the Sch strain from the earlier tested groups. The S_2 stock are descendants of S_1 survivors. In the remaining selected generations occasionally an animal of S_2 was mated to S_3 or S_4, or an S_3 to an S_4 or S_5, if such matings gave promise of greater accumulation of resistance. Progeny from such crosses were recorded as of the selection above the higher numbered parent, that is, a litter from an $S_2\times S_3$ mating was recorded in the S_4 group. Since all matings were made

within closed and closely related groups, extra classification was considered as superfluous.

The groups as considered in this section furnish a general summary of the results of selection. The elements of the selection operating within the generations from the standpoint of individuals are undoubtedly of more vital interest. Their more complete analysis will be found under the section on effects of individuals on selection.

TABLE 6

Percentage mortality and type of dispersion in successive generations of stocks selected for resistance to the standard dose, 5×10^4 organisms. The Sch and Sil groups represent the total number of animals used as controls throughout the six generations.

STOCKS	NUMBER INOCULATED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	L	X^2	P
Sil	108	108	100.0	..	..	..	..
Sch	538	443	82.3	13†	1.19	18.34	0.10
S_1	175	113	64.5	44	1.16	60.15	0.04
S_2	109	50	45.8	27	1.60	69.32	0.01
S_3	123	49	39.8	27	1.08	32.17	0.16
S_4	154	56	36.3	35	1.22	52.15	0.01
S_5	147	48	32.6	35	0.94	31.25	0.49
S_6	105	26	24.7	26	1.11	32.05	0.14
F_1 (Sch×Sil)	106	83	78.3	25	1.05	25.38	0.34
Oc (S*×Sil)	187	70	37.4	47	0.94	41.13	0.64

* Selected animals mated to Silvers.

† Groups tested.

The percentage mortalities in table 6 show a progressive increase in total resistance over the entire period of selection. The greatest effect was obtained in the first and second generations after which the progress was somewhat retarded.

Hybrids from untested Sch×Sil show a mortality approaching that of the more resistant parent strain. The dispersions of mortalities in this F_1 stock and the Sch strain are remarkably constant, being well within that expected from random variability. The high resistance in the F_1 indicates that the parental Sch strain carries inherent factors for resistance since the parental Sil strain is totally susceptible to the dosage used. No Sil animals have survived the standard dose.

In following the type of dispersion through the selected generations, it is worthy of note that in the Sch strain the probability for death is high and uniformly distributed throughout. In the successive generations, S_1, S_2, S_3, and S_4, there is apparently some disturbance of the probability ac-

companying the course of selection. The inbreeding accompanying mating in a closed population should concentrate genetic factors for resistance. If such concentrations are reflected in the tendency of a litter to react as a unit in the test then this tendency for litter differences should be reflected in the statistics describing the generation.

The S_5 and S_6 stocks with a higher concentration of factors for resistance, or, inversely stated, carrying a low probability for death, show the probability uniformly distributed from litter to litter.

The Oc stock in table 6 represents an aggregate of hybrid litters. The Sil strain was used as one parental line. The animals represented by S were from various selected generations. A comparison of the Oc stock with the F_1 stock gives emphasis to the point that genetic factors for resistance have been accumulated. The mortality in the Oc stock is almost identical with that in S_4, 37.4 percent. As yet, no S_6 animals have been mated to the Silvers. Other features of the Oc stock will be discussed under passive immunity and the effects of individuals on selection. In table 7 is given a statistical statement of the proportionate differences between variously selected stocks.

TABLE 7

Tests for independence between successive generations of the selected in addition to the control and out-cross stocks.

STOCKS COMPARED	DIFFERENCES IN PERCENTAGE MORTALITY	X^2	P
Sch and S_1	17.7	22.07	<0.01
S_1 and S_2	18.7	10.29	<0.01
S_2 and S_3	6.0	1.12	0.29
S_3 and S_4	3.5	0.55	0.46
S_4 and S_5	3.7	0.23	0.65
S_5 and S_6	7.9	1.26	0.27
S_2 and S_5	13.2	4.42	0.04
S_4 and S_6	11.6	3.63	0.06
Sil and Sch	17.7	24.50	<0.01
Sil and F_1	21.7	17.58	<0.01
Sch and F_1	4.0	0.66	0.44
Sch and Oc	45.9	134.05	<0.01
Sil and Oc	62.6	112.45	<0.01
F_1 and Oc	40.9	53.20	<0.01

The differences between Sch and S_1, and S_1 and S_2 are statistically significant, indicating that causes other than chance were operating to produce these differences. Taken consecutively from S_2 to S_6 any single interval represents differences easily attributable to variation of random

sampling. However, between S_2 and S_5, and between S_4 and S_6 the differences approach a significant figure supporting the conclusion that over the series of generations the selection has been effective in increasing the level of inherent resistance.

Considering Sch, Sil and their F_1 progeny the difference between the parental strains is significant; between Sil and F_1 is seen statistical identity

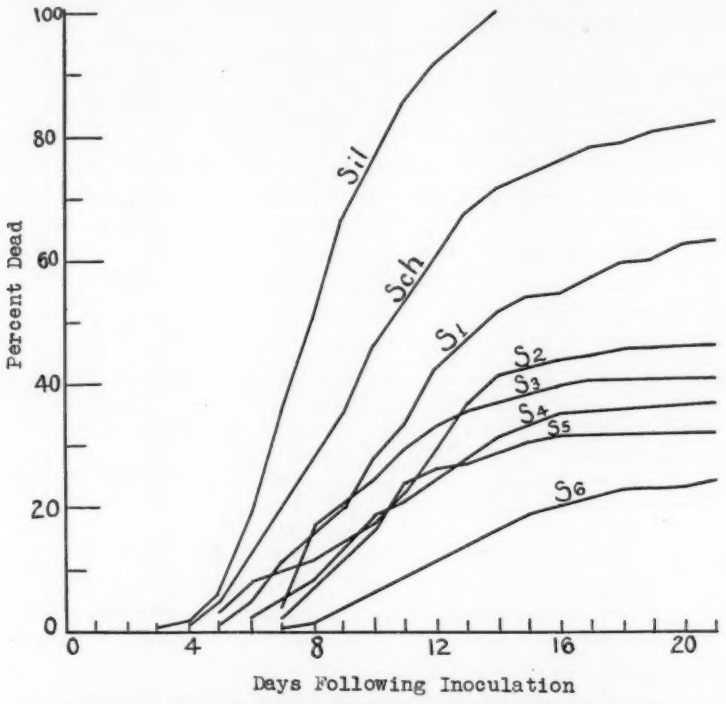

FIGURE 4.—Comparison of death rates of stocks selected for resistance; inoculation with standard dose 5×10^4 organisms.

in their proportionate mortality. The Oc stock shows complete statistical independence from the above three stocks. In each case the P is well beyond 0.01.

A comparison of the percentage mortality rates among the selected stocks is given in figure 4.

The curves show much the same rate of mortality from the S_2 to the S_5

generations. The S_6 group gives a somewhat retarded rate of death, but in order to attach significance to that it would require tests on much larger numbers of similarly selected animals.

In figure 5 is given a comparison of the percentage mortality rates of the F_1 and Oc stocks along with the rates of the Sil and Sch strains.

The similarity of the F_1 reaction to that of the Sch is very striking, again

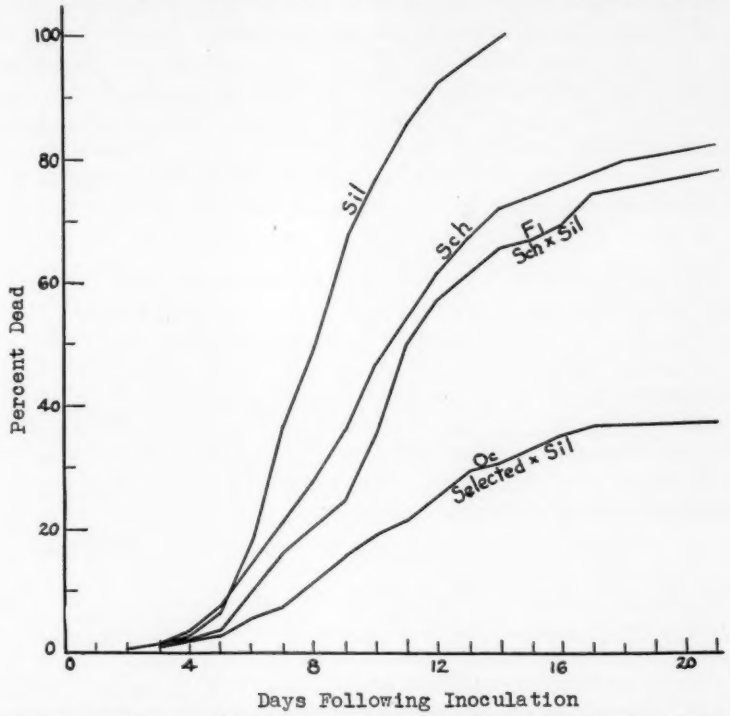

FIGURE 5.—Mortality rates in Sil, Sch, F_1 (progeny of Sil×Sch), and Oc (selected resistant animals×Sil) stocks under the standard dose, 5×10^4 organisms.

lending support to the belief that the Sch stock carries dominant, genetic factors for resistance. The wide separation between the F_1 curve and that of the Sil parental strain also supports the conclusion drawn from data in table 6 that the F_1 progeny inherited their resistance from the Sch parental strain.

The reaction ot the Oc stock, as shown by its mortality curve, has little

in common with that of the Sil parental strain. Its analog is found among the mortality curves of the selected generations in figure 4. The point to be emphasized is that certain individuals after selection for resistance when mated to totally susceptible animals can produce progeny that resist the disease as completely as progeny from matings among the selected animals themselves.

The conclusion that a complex of at least partially dominant genes is largely responsible for resistance would seem justified from the facts that: (1) unselected animals from the Sch strain when mated to Silvers produce progeny practically as resistant as the Sch animals, and (2) selected resistant animals when mated to Silvers produce progeny as resistant as the fourth surviving generation. The fifth surviving generation continues to throw a considerable number of susceptible animals. This indicates that the selected population is still carrying some factors for susceptibility.

PASSIVE IMMUNITY

If passive immunity were considered as largely responsible for the increased resistance resulting from selection, the observations in this experiment could not be used to support this assumption. Males in mammals are not known to be able to transmit acquired immunity to their progeny. If males surviving the disease are mated to females that are from known susceptible stock, and these females themselves have had no direct contact with the disease, the progeny from these matings should be largely susceptible. A part of the Oc stock was made up of progeny from such matings. At test the following results were obtained:

Number tested	*Number dead*	*Percent dead*
160	60	37.5

The argument for passive immunity in this case is invalid, for the resistance in the 160 Oc mice is practically equivalent to that in the S_4 selected resistant generation, that is, 36.3 percent.

If a latent infection capable of rendering mice highly resistant were at large in the propagation laboratory at any time during the three years of the experiment, it would seem remarkable that some animals of the Sil or Wf strains had not acquired the protection before coming to test.

Furthermore, four Sil females, each having first produced an Oc litter, were mated to Sil males. Fourteen of the Sil offspring from these matings came to test and none showed indifference to the infection. A comparison of the progeny of the four Sil females shows the following:

Stock	*Number tested*	*Number dead*	*Percent dead*
Oc	17	6	35.39
Sil	14	14	100.00

If passive immunity were operating, the progeny from selected resistant females on Sil males should have a marked advantage over the progeny from the reciprocal matings. Progeny from six selected females mated to Sil males were tested, and gave the following reaction:

Number tested	*Number dead*	*Percent dead*
27	10	37.0

While the number in this group is too small to carry much weight, the reaction suggests no real advantage in resistance over the progeny from the reciprocal crosses.

The fact that the young mice in all cases were separated from their mothers approximately four weeks before testing would seem to be sufficient ground for believing that any temporary passive immunity derived from the mother had been lost before the time of inoculation.

From the above considerations it is concluded that passive immunity has played a minor, if any, part in the resistance observed in this study.

AGE, WEIGHT, AND SEX AS FACTORS IN RESISTANCE

Experience with 2901 mice at test precludes the assumption that age within limits concerned in this study, or weight as incidental to age, or sex, has played any critical part in resistance. A summary of these features relative to certain salient groups is given in table 8.

The S_1 generation was chosen as an example of the early stages of the selection and the S_8 is representative of the advanced selection. In the S_1 males as shown in table 8 there is an apparent tendency for a higher percentage of the younger and lighter males to die. This is not apparent in the corresponding female group, however.

TABLE 8

Showing average age, weight, and days to death in S_1 and S_8 male and female groups.

STOCK	SEX	NUMBER	AVERAGE AGE IN DAYS	AVERAGE WEIGHT IN GRAMS	AVERAGE DAYS TO DEATH
S_1	♂♂	Survive 31	60.3±0.64	19.2±0.31	..
		Dead 60	56.7±0.45	18.4±0.17	11.8±0.28
	♀♀	Survive 31	58.4±0.66	16.7±0.24	..
		Dead 53	58.5±0.75	16.5±0.18	11.1±0.46
S_8	♂♂	Survive 49	49.9±0.94	18.9±0.18	..
		Dead 23	62.8±0.60	19.1±0.26	11.4±0.47
	♀♀	Survive 50	60.8±0.47	16.5±0.14	..
		Dead 25	60.7±1.00	17.2±0.19	10.8±0.39

In the S_5 stock the apparent differences in S_1 are reversed. Neither in these two groups nor in any other of the six selected generations was there any marked advantage in resistance noted because of greater age or weight.

Neither has sex given any indication of association with resistance in any group. In the S_1 and S_5 generations the proportionate mortalities in the sex groups are practically identical.

In the Sil stock where, presumably, factors for resistance are absent the effects of age and weight can be spoken of only as they are associated with rate of mortality. The correlation coefficient between age and days to death is 0.017 and that between weight and days to death is 0.299. Between weight and age there is an r of 0.134. If one refers to FISHER's (1930, p. 176) table for significance of correlation coefficients, the r of 0.299 is significant. The heavier animals in the Sil strain tend to live slightly longer than the light ones.

Among the Oc animals which were sired by selected males it might be expected, since the male is heterozygous for sex, that the association of resistance with maleness or femaleness would have opportunity of expression. The 160 Oc mice from selected resistant sires show:

	Number tested	*Number dead*	*Percent dead*	*Percent difference*	X^2	*P*
♂♂	87	31	35.6	4.1	0.43	0.65
♀♀	73	29	39.7			

The proportionate difference in mortality between males and females in this group is too small to suggest any advantage of one sex over the other.

INBREEDING IN THE SELECTED GROUPS

Mating within a closed population from generation to generation should automatically increase the homozygosity of the group. The inbreeding and relationship coefficients in the selected generations have been calculated according to the method of WRIGHT (1923).

The surviving animals from the earlier tests on the Sch strain were mated together. Their surviving progeny in turn were intermated, and the whole series of related animals through six generations was designated as group I. Another similar series, group II, was developed from Sch survivors that were at test later than the progenitors of group I. Group II was also mated entirely within itself and includes animals from the S_1 to S_5 generations.

The percentage mortality in group I in each consecutive generation with the average inbreeding is given in table 9. The percentage dead from generation to generation does not decrease uniformly, while the inbreeding increases at a quite uniform rate.

TABLE 9

Percentage mortality and percent inbreeding per generation in group I.

GENERATION	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	AVERAGE PERCENTAGE INBREEDING
S_1	30	16	53.3	0.0
S_2	46	19	41.3	14.2
S_3	65	30	46.0	23.9
S_4	95	31	31.5	31.5
S_5	119	38	31.9	33.3
S_6	105	26	24.7	43.0

The distribution of mortalities in each generation relative to the amount of inbreeding is listed in table 10. Here it will be noted that there is a considerable range of inbreeding in each generation after the S_1.

TABLE 10

Generations in group I showing percentage mortality for each ten percent range of inbreeding.

	S_1		S_2		S_3		S_4		S_5		S_6	
PERCENT INBREEDING	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED
50–59	..	..	..	..	..	..	..	..	..	..	35.9	(26)
40–49	..	..	..	..	75.0	(4)	37.0	(27)	26.8	(41)	19.5	(46)
30–39	..	..	28.5	(14)	40.0	(10)	25.0	(28)	31.5	(38)	23.9	(17)
20–29	..	..	..	..	41.4	(41)	56.2	(16)	40.0	(15)	37.5	(16)
10–19	..	..	42.8	(14)	12.2	(7)	26.3	(24)	36.0	(25)	..	..
0– 9	53.3	(30)	50.0	(18)	100.0	(3)	..	..	..	..	..	..

In the second series of matings designated as group II the same general trends and relationships are apparent as were noted in group I. The summaries of the group II percentages are given in tables 11 and 12.

TABLE 11

Percentage mortality and percentage inbreeding in the generations of group II.

GENERATION	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	AVERAGE INBREEDING
S_1	37	19	51.0	0.0
S_2	63	31	49.2	8.3
S_3	58	19	32.7	23.9
S_4	59	25	42.4	32.8
S_5	28	10	35.7	29.8

In table 12, as was pointed out in table 10, there is considerable variation in the inbreeding of any one generation.

TABLE 12

Distribution of inbreeding in consecutive generations relative to mortality in the generations of group II.

PERCENT INBREEDING	S_1		S_2		S_3		S_4		S_5	
	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED
50–59	..	..	..	..	..	..	..	..	33.3	(3)
40–49	..	..	..	..	..	..	33.3	(6)	..	..
30–39	..	..	..	..	38.4	(13)	38.4	(26)	..	..
20–29	..	..	..	..	31.1	(45)	48.1	(27)	36.0	(25)
10–19	..	..	42.8	(42)	..	..	..	..	..	..
0– 9	51.6	(37)	61.9	(21)	..	..	..	..	..	..

If there were a tendency for higher inbreeding as such to be associated with lower resistance the higher mortality percentages would be distributed through the upper ranges of the tables. This or the inverse situation is not apparent. Therefore, it must be concluded that the amount of inbreeding as expressed by the coefficients is no direct index of resistance or susceptibility in the animals used in this experiment. However, it is believed that the inbreeding and the relationship incidental to it are useful descriptive measures in the analysis of the uniformity of any group of animals.

The slight fall in the average inbreeding in the fifth generation of group II as shown by table 11 indicates only that the less highly inbred animals of the preceding generation have thus far had progeny at test.

The whole upward trend of the inbreeding coefficients in the two groups gives a fair picture of the gradual tendency toward homozygosity. If the resistance were heightened by homozygous factors then the course of selection should have increased the homozygosity above that of the average estimated figure. If selection were favoring the heterozygous individuals, on the other hand, then the average estimated coefficient of inbreeding for any generation would be higher than the actual homozygosity in that generation.

As yet there is no means of measuring the whole genetic make up of an animal. However, by a gradual process of narrowing the chances for heterozygosity by breeding within a closed group, along with rigid selection, there should be a tendency toward a more uniformly resistant population. The selection, however, is incomplete if the individual's ability to survive

is the only criterion used. The manner in which individual performance coupled with that of progeny and progenitors has been used in the selection will be pointed out below.

THE INFLUENCE OF INDIVIDUALS IN SELECTION

Among the Sch survivors that were the parents of the S_1 generation in group I, a male, Sch 162, and a female, Sch 217, were from separate sire-daughter matings. This male and female were 25 percent inbred and not more closely related than the average individuals of the Sch strain. The reactions of the progeny of these two individuals supported the belief that already a considerable concentration of factors for resistance had taken place. Their progeny predominate in the S_1 generation. Only four unrelated S_1 females were mated into the group to produce the S_2 generation.

Three S_1 males, $S_1$1, $S_1$3, $S_1$8, all brothers, from ♂ Sch 162 × ♀ Sch 217, sired the entire S_2 generation of group I. A summary of the reaction of the progeny of each of these males is given in table 13 along with the average inbreeding coefficient of each progeny group under F_o and the average relationship coefficient of the sire to his progeny under R_{so}.

TABLE 13

Percentage mortality of progeny of S_1 males with average inbreeding and average relationship coefficients.

SIRE	DAMS	NUMBER INOCULATED	PERCENT DEAD	F_o AVERAGE INBREEDING	R_{SO} AVERAGE RELATIONSHIP OF SIRE AND PROGENY
$S_1$1	Selected ♀♀	21	33.3	19.2	60.8
	Sil ♀♀	5	40.0	0.0	50.0
$S_1$3	Selected ♀♀	14	57.1	8.9	56.1
	Sil ♀♀	7	28.6	0.0	50.0
$S_1$8	Selected ♀♀	11	36.4	11.4	57.6

The data in table 13 show that there was some homozygosity possible in the S_2 progeny although the S_1 parents were not inbred. The homozygosity came from the two inbred grandparents by brother-sister and half brother-sister matings.

There were three litters in the S_2 generation from brother-sister matings, one from each of the S_1 males. The 14 young in the three litters were 31.25 percent inbred. This rapid concentration of factors seemed not to hinder resistance for 10 of the 14 survived the test. However, of the five survivors

of the litter of 8 sired by ♂ $S_1$1, two males were discarded at the close of the test period and the remaining two males and one female proved sterile in all matings. The remaining two litters consisted of a male and female sired by $S_1$3, and one male and three females sired by $S_1$8. The two individuals from ♂ $S_1$3 were designated as ♂ $S_2$87 and ♀ $S_2$88. Both survived the test. Of those from ♂ $S_1$8 the three females survived. Male $S_2$87 became the dominating sire of the following generations in group I through the good resistance of this progeny and the favor which certain of them received in selection.

That certain animals can resist the disease and yet be of no use in the accumulation of resistance can be illustrated by the performance of progeny from ♂ $S_2$8. When mated to ♀ $S_3$14 he gave a litter of four. All four died at test. At the same time he produced two litters by Sil females, 9 Oc progeny. Eight of these died at test.

It was decided to make further tests of the apparent susceptibility of the progeny of ♂$S_3$8 and ♀ $S_3$14. A second litter was produced, one male and three females. Before these were mature the sire had died so the litter was not tested. The dam and three daughters were mated to the young male, and progeny coming to test gave:

Number tested	*Number dead*	*Percent dead*
28	19	67.8

Three males and nine females of the brood of ♀ $S_3$14 were not tested; matings among this group gave:

Number tested	*Number dead*	*Percent dead*
27	17	62.9

These results of negative selection show that although some individuals can resist the disease they carry heritable factors that tend to render some of their progeny highly susceptible. It would be presumptious to suppose that all of the possibilities for detrimental combinations had been eliminated from the resistant animals after three or even six generations of selection.

That male $S_2$87 carried high potentialities for heritable resistance can be concluded from the list of his progeny given in table 14.

It will be noted that resistance in the progeny by S_3 females is better than that of the progeny by the S_2 females. Also, the inbreeding of the progeny and the relationship of the progeny to the sire have increased. Stated in terms of mean percentages these show:

Dams of generation	*Number of progeny tested*	*Percent dead*	*Average F_o*	*Average R_{so}*
S_3	18	50.0	31.6	74.5
S_3	28	25.0	41.5	77.9

Perhaps no better description of the importance of ♂ $S_2$87 in the group I population can be given than by noting the increase of his average relationship to each consecutive generation. The coefficients are given in table 15 along with the relationship of a male from each generation having the highest coefficient other than ♂ $S_2$87. The male listed in each case is

TABLE 14

Progeny of ♂ $S_2$87 from various females showing number dead, percent inbreeding, and relation of progeny to the sire.

DAMS	NUMBER TESTED	NUMBER DEAD	F_o PERCENT INBREEDING OF PROGENY	R_{so} RELATIONSHIP OF SIRE TO PROGENY
$S_2$74	4	0	23.4	69.9
$S_2$92	2	2	31.2	73.8
$S_2$91	8	4	31.2	73.8
$S_2$88	4	3	40.6	80.9
$S_3$20	11	0	32.0	74.2
$S_3$10	3	3	44.5	74.2
$S_3$42	6	2	48.4	81.7
$S_3$43	8	2	48.4	81.7
$S_4$4	7	3	38.3	77.2
Sil ♀ ♀	16	5	00.0	50.0

the one having the most offspring in the following generation. Also the relation between the listed male and ♂ $S_2$87 is given. The coefficients of relationship between males and generations in the table are in all cases relative to the group of individuals which produced progeny and not to the generation as a whole.

The relationship coefficients of ♂ $S_2$87 to the reproducing individuals show more than a doubling from the S_1 to the S_5 generation. This increase in the coefficient values indicates that factors carried by ♂ $S_2$87 have become 67.5 percent of all the factors carried by the reproducing individuals in the S_5 generation.

The males from consecutive generations as listed in table 15 were all more closely related to ♂ $S_2$87 than brothers in a random bred population. Male $S_4$5 was a son of ♂ $S_2$87 × ♀ $S_3$20. He sired eleven of the twenty-seven litters in the S_5 generation group.

TABLE 15

The relationship of ♂ $S_2$87 to the reproducing group in five generations, and the relationship between certain males to their own generation and to ♂ $S_2$87.

GENERATION*	S_1	S_2	S_3	S_4	S_5
Average R of generation to ♂ $S_2$87	32.2	42.8	51.6	62.7	67.5
Selected sire of each generation	♂ $S_1$1	♂ $S_2$69	♂ $S_3$31	♂ $S_4$5	♂ $S_5$3
Selected sires' relationship to his own generation	42.7	28.9	37.6	51.7	62.3
Selected sires' relationship to ♂ $S_2$87	54.5	35.4	36.1	57.7	70.1

* The relationship of any sire to the generation refers to that group of individuals which produced progeny.

It was evident that certain S_3 litters, closely related to ♂ $S_2$87, were at an advantage in the tests. Matings in the fourth and fifth generation, therefore, were made to favor a concentration of factors carried by individuals from such litters. This concentration of factors is reflected in the increased relationship of ♂ $S_2$87 to the S_5 over his relationship to the S_4. He had sired only one litter listed under S_5 while he had sired five litters listed under S_4.

Examination of the relationships of ♂ $S_2$87 shows: ♂ $S_4$5 mated to a sister, ♀ $S_4$6, produced two S_5 litters, and with another sister, ♀ $S_4$8, he gave one litter. Of the 13 progeny, 10 survived. Male $S_5$3 listed in table 15 was from this group. Male $S_5$4 was a full brother of ♂ $S_5$3 and ♂ $S_5$1 was from ♂ $S_4$5 × ♀ $S_4$6. The three S_5 males, $S_5$1, $S_5$3, and $S_5$4, sired 14 of the 26 litters of the S_6 generation that have been tested. This inbred line of descendants kept a high concentration of the factors of ♂ $S_2$87 in the S_6 generation. The average coefficient of relationship between ♂ $S_2$87 and the S_6 generation is 51.5 percent. This indicates that although he sired none of the S_6 animals he still had the genetic status of sire to that generation.

It is believed that through the detection of the inherent potentialities for resistance in ♂ $S_2$87 and through the subsequent concentration of those potentialities it has been possible to maintain and increase the resistance through the later generations. Continued inbreeding in a naturally crossbred species is generally considered as a precarious practice with no other demands on the individuals than reproduction. In this study the additional requirements have been ability of the animal to resist the disease as well as to transmit the factors that make for resistance. The conclusion seems justifiable that animals having these requirements have been found and that concentration of the underlying heritable factors has been carried on as rapidly as was allowable with the population at hand.

In group II the same tendency for the generations to follow the line of certain males is evident. A detailed analysis of group II would be a needless repetition of the methods explained above. The increase of the inbreeding coefficients as shown in table 11 implies that there has been a gradual increase of the relationship between individuals, and a gradual concentration of factors for resistance has been carried on by mating progeny from individuals that have proved their worth by giving a high percentage of resistant offspring.

SUMMARY

In this investigation a total of 2901 mice have been inoculated intraperitoneally with controlled doses of living *Salmonella aertrycke*.

A standard dose of 5×10^4 organisms was used as a basis of selection for resistance after the reactions of three different laboratory strains of mice had been observed under various sized doses. Animals from four other distinct strains of mice have been tested with the standard dose.

The seven strains of mice and their respective percentage mortalities under the standard dose were: Schwing albino (82.3), Bagg albino (80.4), Strong dark-brown (90.7), Little dilute-brown (96.4), Short-eared dilute-brown (84.7), English silver (100.0), and White-faced (100.0).

Selective breeding for resistance was continued through six successive generations using survivors of the Schwing strain as the beginning of the selection. The percentage mortality in the successive selected generations was as follows: Schwing (82.3), first (64.3), second (45.8), third (39.8), fourth (36.3), fifth (32.6), and sixth (24.7).

Untested Schwing mice were mated to Silvers and the F_1 progeny at test gave a mortality percentage of 78.3. Selected resistant animals mated to Silvers gave progeny which showed only 37.4 percent dead at test. These results with the hybrid generations together with a consideration of the progressive increase in resistance in the six selected generations justify the conclusions: a marked concentration of heritable factors for resistance has been obtained; this resistance depends largely on a complex of at least partially dominant genetic factors.

It is shown that the possible effects of passive immunity as an explanation of increased resistance could be of no importance in the resistance observed.

Within the limits concerned in this study, age at the time of testing and likewise weight have not played a critical part in resistance. One sex shows no advantage over the other at test.

Coefficients of inbreeding and relationships have been calculated for the selected groups and used in the report as a descriptive estimate of genetic uniformity.

It has been pointed out that phenotypic resistance of the individual animal alone cannot be used as the only criterion for selection. An intimate knowledge of the individuals in relation to their progenitors and progeny is necessary in order to make matings that will maintain and increase the high level of resistance over a series of generations in a closed population.

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TABLE OF CONTENTS

PAGE

FISHER, R. A., IMMER, F. R., and TEDIN, OLOF, The genetical interpretation of statistics of the third degree in the study of quantitative inheritance 107

POOLE, CHARLES F., Pollen grain studies as an indication of fertility in hybrids 125

REDFIELD, HELEN, A comparison of triploid and diploid crossing over for chromosome II of *Drosophila melanogaster* 137

BIDDLE, R. L., The bristles of hybrids between *Drosophila melanogaster* and *Drosophila simulans* 153

EAST, E. M., Studies on self-sterility. IX. The behavior of crosses between self-sterile and self-fertile plants 175

SCHOTT, RALPH G., The inheritance of resistance to *Salmonella aertrycke* in various strains of mice 203

SIXTH INTERNATIONAL CONGRESS OF GENETICS

ITHACA, NEW YORK, AUGUST 24-31, 1932

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[This number of GENETICS (17: 107–130) was issued March 20, 1932]